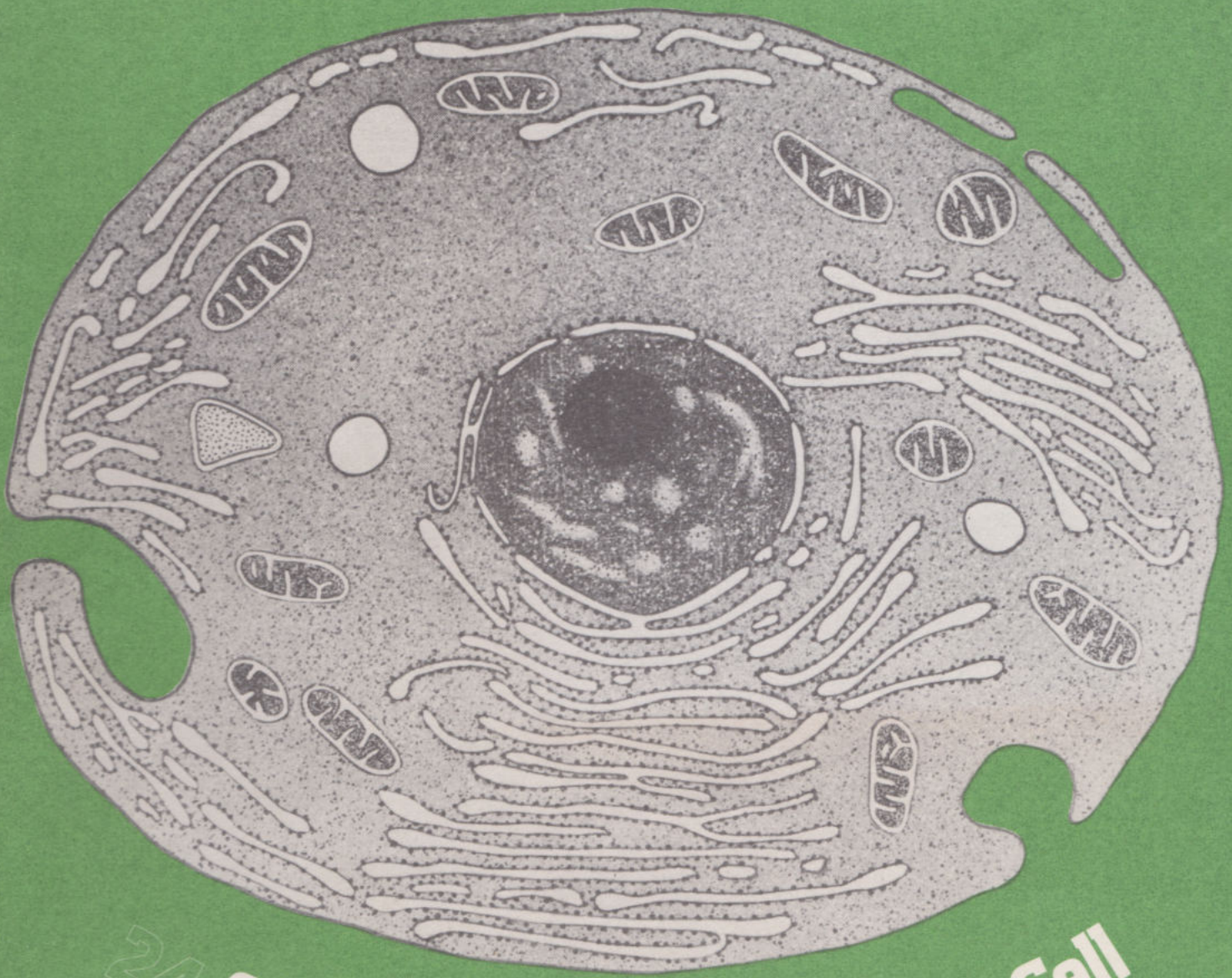
S101 Units 23 and 24

THE OPEN UNIVERSITY

Science : A Foundation Course

23 Cell Structure and the Chemical Components of Cells



24 Chemical Reactions in the Cell

The Open University
Science: A Foundation Course

Unit 24
Chemical reactions in the cell

Prepared by the Science Foundation Course Team

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Study Guide

This Unit builds on the text of the previous Unit, to which you may need to refer. It aims to give you an idea of the *principles* behind the web of chemical reactions taking place within the cell. Do not be put off by the apparent complexity of this network of reactions. You are expected to remember principles rather than details. It is therefore particularly important to read the Study comments, and to check the Objectives at the end of each Section.

Sections 1–3 provide background introductory material and are comparatively straightforward. Section 4 sets out the principles of metabolism, and is therefore very important. Section 5 is the meatiest Section; pay careful attention to the study comment that precedes it. It takes one set of reactions—those concerned in the oxidation of glucose and other nutrients—and studies them in detail. If pressed for time, you could omit Section 5.4.2 on amino acid breakdown, though you will then be unable to fulfil all Unit Objectives.

Section 6 considers briefly the reactions concerned in synthesizing rather than degrading molecules in the cell. If very pressed for time, you could omit the Section on photosynthesis (6.3) although again, this relates to several Unit Objectives.

The final Section is rather different. It moves away from metabolic pathways to consider one of the key processes in cell chemistry—the mechanism of energy conservation in mitochondria and chloroplasts.

The television programme (TV 24) relates to both this Unit and to Unit 25. It illustrates some of the techniques used in studying macromolecules of all kinds, although in this case we are examining deoxyribonucleic acid (DNA).

The Radio programme (R 12) on metabolic pathways is designed to clarify the Main Text of this Unit.

1 Introduction

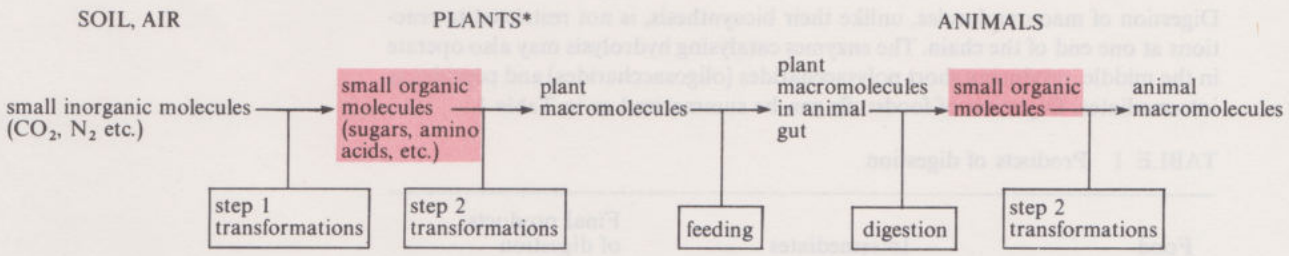
Living organisms have developed the ability to perform and control an enormous number of highly specific chemical reactions. They have also developed the ability to harness the sun's energy and store it in such a way that it can be reused in small quantities. In the last Unit you saw how the specificity of biological reactions derives from the way the macromolecules of the cell are built up. In this Unit you will see how both energy and materials enter into cell chemistry. You will study some of the transformations they undergo there, and see how these may be controlled by one particular class of macromolecule—the protein catalysts called enzymes.

2 Food chains

Examples of transformation of energy and materials are all around us. An oak tree grows from an acorn, a slice of damp bread lying around becomes covered in a fine mat of fungus, apparently from nowhere, but in reality from growth of minute airborne fungal spores. The tissues of our own bodies are constantly being replaced from raw materials in the food we eat. (How is it that, as the poet Walter de la Mare put it, 'whatever Miss T eats turns into Miss T'?)

In our own case, the sources of raw materials for body building are fairly obvious. With the oak tree, the sources are largely invisible, as small inorganic molecules present in the soil and the air. Only plants and bacteria can cope with these small-molecule sources of raw material, because only they have the necessary enzymes. Animals join in the food chain only after the small molecules have been converted into macromolecules, by eating the plants. This *food chain* is outlined in Figure 1. It is another way of looking at the food webs described in Unit 21.

food chain



* And associated micro-organisms, e.g. those capable of nitrogen fixation

FIGURE 1 Food chain. Note particularly the position of small organic molecules shaded in pink. Much of this Unit is concerned with their fate in the cell.

At the beginning of the chain is a two-step transformation of materials within the plant body. The very small inorganic molecules like nitrogen* and carbon dioxide in the air, or nitrate ions in the soil are first converted to organic chemicals. These are the small organic molecules described in Table 4 of Unit 23, that is, sugars, amino acids, etc. In the next stage the plant elaborates these into macromolecules.

Plant macromolecules taken in by the animals during feeding are the starting point for transformations that take place within the animal body. Digestion (discussed below) is the first step. Here the macromolecular foodstuffs are broken down again to small organic molecules. These can be converted to macromolecules of the animal body in much the same way as they were converted to plant macromolecules in step 2 of the food chain.

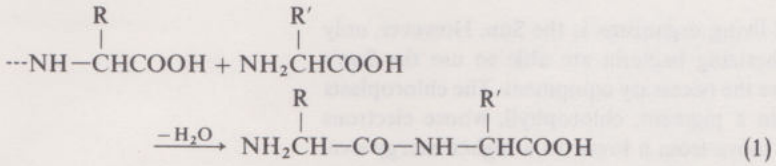
It is only the first transformation—inorganic molecules to organic ones—that animals are unable to perform. For the second stage—organic molecules to macromolecules—they have the same kind of equipment as plants.

2.1 Digestion

Breakdown of food in the animal body is the process known as digestion. It is essentially the reverse of the condensation reactions by which small organic molecules are linked together, as you have seen in Unit 23. In digestion, the elements of water are reinserted, in a series of hydrolysis reactions.

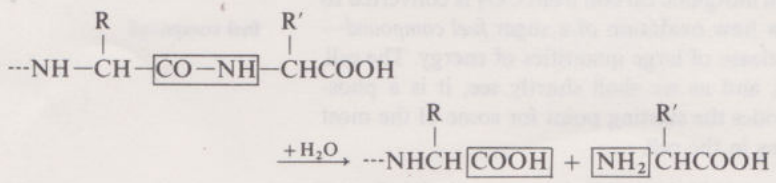
If equation 1 shows a *condensation* reaction during the synthesis of protein, write down the corresponding equation to demonstrate what happens in the *digestion* of this protein (--- represents the end of a long polypeptide chain).

digestion



(1)

Digestion is represented by:



In the gut, this reaction is catalysed by proteases (protein-hydrolysing enzymes) such as pepsin. Other digestive enzymes hydrolyse polysaccharides to sugars, and fats to glycerol and fatty acids. These reactions are essentially the reverse of the condensations described in Unit 23, equation 5 (Section 9.2) for fats and Figures 9 and 10 (Section 10.1) for polysaccharides and proteins.

* Strictly, nitrogen fixation (the process by which atmospheric nitrogen is converted to ammonia) takes place not in plants but in micro-organisms closely associated with them.

Digestion of macromolecules, unlike their biosynthesis, is not restricted to reactions at one end of the chain. The enzymes catalysing hydrolysis may also operate in the middle, producing short polysaccharides (oligosaccharides) and peptides as intermediates. Digestion of foodstuffs can be summarized as in Table 1.

TABLE 1 Products of digestion

Food	Intermediates	Final products of digestion
proteins	peptides	amino acids
fats		fatty acids + glycerol
polysaccharides	oligosaccharides	sugars

The small-molecule products of digestion have still to reach the cells that need them. In simple animals, they travel there just by diffusion*. In higher organisms there are specialized transport systems, blood, for example, which carry nutrients from the gut and deliver them to within micrometres of the membrane of each cell. The final stage, passage across the cell membrane, may require special carrier mechanisms, or may again occur by simple diffusion.

3 Energy in biology

3.1 Energy-consuming processes of living organisms

None of the characteristic activities of living organisms that were listed in Unit 18, Section 1, could be carried out without some input of energy. All living organisms are capable, for example, of constructing complex molecules from simple ones for body building, that is, growth, and reproduction. At the molecular level, this is known as biosynthesis, a topic we shall explain shortly. All living organisms can also pump ions (such as Na^+) out of the cell against a concentration gradient*. Even the cells of organisms surrounded by sea water are often less salty than the sea itself. This ion pumping requires energy. Finally, many of the animals can move around, and this muscular activity also requires energy.

3.2 Energy chains

The primary source of energy for all living organisms is the Sun. However, only green plants and certain photosynthesizing bacteria are able to use the Sun's energy directly, because only they have the necessary equipment. The chloroplasts of green plants, for example, contain a pigment, chlorophyll, whose electrons become excited by light energy, and move from a lower to a higher energy level (see Units 10 and 11). The return of these electrons to lower energy levels is accompanied by a series of chemical reactions. The most important of these is the carbon-dioxide fixation step, in which inorganic carbon from CO_2 is converted to sugar. In Unit 15, Section 4, you saw how oxidation of a sugar *fuel compound*—glucose—was accompanied by the release of large quantities of energy. The cell, too, uses sugar as a fuel compound, and as we shall shortly see, it is a phosphorylated form of glucose that provides the starting point for some of the most important energy-producing reactions in the cell.

fuel compound

Although glucose is one of the commonest biological fuel compounds, it is far too soluble to be stored in large quantities. *Fuel-storage compounds* are either fats, or polysaccharides such as glycogen or starch. These last are formed by polymerization of glucose. Whatever their nature, all fuel-storage compounds are ultimately derived from the sugar products of *photosynthesis*. Equation 2 sums up this relationship.

fuel-storage compound

photosynthesis

$$CO_2 + H_2O \xrightarrow[\text{chlorophyll + LIGHT}]{\text{photosynthesis}} \text{sugar} \longrightarrow \text{fuel-storage compound} \quad (2)$$

* See third footnote on p. 35 for an explanation of these terms.

One advantage of fuel-storage compounds is that there is no need to rely directly on the Sun. Animals can get their energy indirectly from the plants, and the plants themselves can survive considerable periods of darkness, and can load up their offspring (seeds) with sufficient fuel to survive and grow until they have developed their own chloroplasts.

3.3 Respiration

The breakdown of fuel compounds in the cell is a process common to all living organisms and is known as respiration. The oxidation of glucose for example, can be expressed by the equation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \qquad (3)$$

The equilibrium constant for this reaction (see Units 14 and 15) is very high, so you might well expect glucose to disintegrate spontaneously into CO_2 and H_2O. Yet glucose, like the closely-related sugar sucrose used in everyday cookery, is perfectly stable at room temperature. The reason for this is the exceedingly high activation energy of the reaction shown in equation 3. Without a catalyst therefore, glucose oxidation proceeds invisibly slowly.

In the body, glucose oxidation is catalysed by enzymes, the activation energy is lowered, and the reaction rate is greatly increased. This, however, introduces another problem. Glucose oxidation yields $2\,500\,kJ\,mol^{-1}$. When the reaction takes place in the air, all this energy is released in a single step, in the form of heat. If the same thing were to happen in the living cell, its structure would be quite disrupted. Furthermore, there is no single biochemical process that could make use of so much energy. But in biological systems, this energy is released slowly, by means of a whole series of small steps. Each step is catalysed by an enzyme, and each may be linked directly or indirectly to the promotion of an energy-requiring reaction.

respiration

The process of *respiration* describes, at the molecular level, what is happening to the oxygen taken up and the carbon dioxide released during, for example, the breathing of higher animals. The overall reaction can be represented by the same equation as the combustion of glucose outside the cell, that is, equation 3, but because cellular respiration takes place in a series of enzyme-catalysed reactions, the energy released in each step can be made use of by the cell.

In the rest of this Unit we shall describe first the breakdown of fuel compounds in the cell, and then the use of energy released in just one type of energy-consuming process, biosynthesis.

3.4 Summary of Section 3

1 All living organisms are linked to the sun via an energy chain which may be represented as follows:

$$\text{Sun} \xrightarrow{\text{photosynthesis}} \text{fuel-storage compound} \xrightarrow{\text{respiration}} \text{energy}$$

2 *Fuel-storage compounds* are insoluble compounds formed from the products of photosynthesis. Often they are polymerized forms of glucose (for example, starch or glycogen). These must be hydrolysed back to glucose before respiration begins. (Fats are another type of fuel-storage compound. Protein may also be used as a source of fuel compounds, although this is never its main function in the cell.)

3 *Photosynthesis* can be performed only by organisms with specialized light-trapping pigments such as chlorophyll, that is, green plants and certain bacteria.

4 *Respiration* is the controlled oxidation of glucose and other fuel compounds. It yields energy in a form that can be used by the cell for its various energy-consuming reactions. It occurs in all cells, both plant and animal, and can be represented by the equation:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O \qquad (3)^*$$

3.5 Objectives of Sections 1–3

Now that you have studied Sections 1–3 you should be able to:

(a) Explain, in terms of the chemical reactions that can be performed by different organisms, why animals are dependent on plants.

(b) Explain what is meant by the terms digestion, respiration, fuel compound, fuel-storage compound.

(c) List examples of energy-requiring and energy-producing reactions in the cell.

(d) Describe in words, the chemical changes resulting from the digestion of macromolecular foodstuffs.

To test your achievement of these Objectives, try the following SAQs.

SAQ 1 (*Objective* (*a*)) What are the processes whereby (i) plants and (ii) higher animals obtain the fuel-storage compounds they require for respiration?

SAQ answers are on p. 38.

SAQ 2 (*Objectives* (*b*)–(*d*)) Classify the following statements as either TRUE or FALSE.

(a) Respiration is a vital characteristic of all living organisms.

(b) Digestion is a process by which macromolecular foodstuffs are degraded to their inorganic component molecules.

(c) Biosynthesis is an energy-requiring process, in which small molecules are built up into complex cellular components.

(d) Muscular activity produces heat, and is therefore one of the cell's energy-producing reactions.

4 Principles of metabolism

4.1 Biosynthesis and degradation

Once they have arrived in the cell, the small organic molecules whose origins we have just described are faced with an enormous array of enzymes. Each catalyses a different step in a series of chemical transformations, which lead ultimately to either degradation or biosynthesis.

degradation

Degradation of nutrients is essentially the reverse of step 1 in the food chain (see Figure 1) namely, small organic molecules (nutrients) ——→ small inorganic molecules. Degradative reactions, such as those involved in the oxidation of fuel compounds, tend to yield energy rather than to consume it, and this is one reason why they are so important in the economy of the cell. The other reason is that the intermediates formed during their partial breakdown can be put together again in different ways, to form different molecules. In this way, the early products of a degradative pathway may provide the starting compounds of a biosynthesis. The only degradation we shall consider in detail here is that of *glucose*, one of the major energy-releasing processes in the cell. The oxidation of *fatty acids* to carbon dioxide and water is another important source of energy, and also provides the carbon atoms for biosyntheses; the breakdown of *proteins* is an important source of nitrogen compounds, as well as—in conditions of starvation—a further source of energy.

biosynthesis

Biosynthesis, the process of building small organic molecules into the complex molecules of the cell, is one way in which this energy is used up. The bulk of the complex molecules are the macromolecules—protein, nucleic acid, lipid—described earlier. However, biosynthesis also produces numerous specialized substances like lignin (which is responsible for hard woody properties of higher plants) and pigments (for example, the brilliant colours of flower petals and the vital green pigment chlorophyll.) Just what an organism can synthesize depends on the enzymes it has, and this is determined by its genetic make-up.

Metabolism is simply the sum of all reactions—degradative or biosynthetic—that may occur in the cell. Despite its apparent complexity, it is no more than a collection of *metabolic pathways* or series of chemical reactions, in which each step is catalysed by an enzyme. Each pathway starts with a particular carbon compound and ends with a different one. Along the way, the carbon compound passes through a series of intermediates, some of which may be channelled into or out of branching pathways.

metabolism

metabolic pathways

4.2 Group-transfer molecules

In the steps converting one intermediate of a metabolic pathway to another, small groups of atoms may be added or removed. This brings us to a highly important feature of metabolism, and one that has no exact parallel in chemical reactions occurring outside the cell: the role of group-transfer molecules.

We have already had examples of reactions in which a small group of atoms (H_2O) is added to a carbon compound in the body. This is in the hydrolysis of macromolecules. These reactions are exceptional, in that the molecule of water used up during reaction comes from the surrounding medium, and is not attached to any carrier. The same is not true of other groups of atoms that participate in metabolism, for example, NH_2, 2H and PO_3H_2. They arrive at the enzyme active site attached to a special *group-transfer molecule* which—just like the substrate of the reaction—fits into a specific area in the enzyme active site (Figure 2). Just as

group-transfer molecule

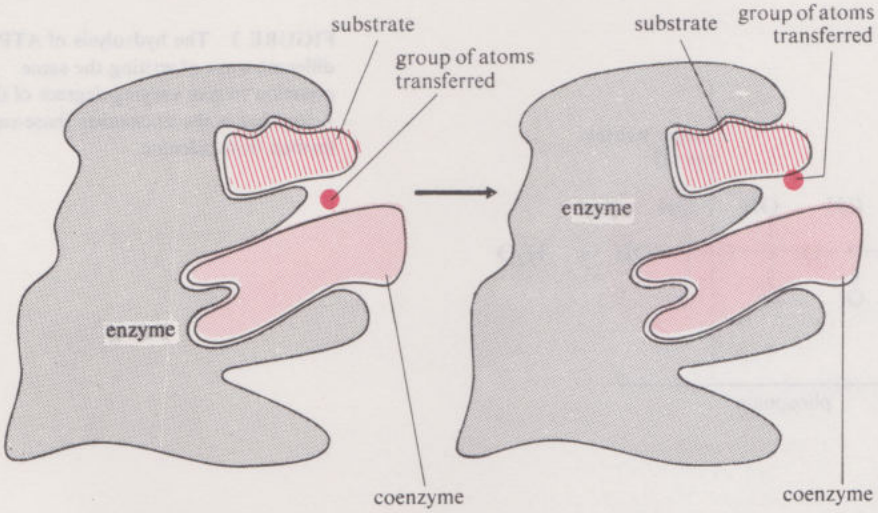


FIGURE 2 Binding sites on the enzyme surface for substrate and coenzyme (group-transfer molecule). Notice how both molecules fit into regions of complementary shape in the enzyme active site.

the substrate must be carefully aligned with respect to the catalytic amino acids in the active site (see Unit 23, Section 11.3), so the group-transfer molecule must be carefully aligned with respect to substrate. Transfer of the small group of atoms between it and substrate can then readily take place, catalysed by the enzyme*.

Group-transfer molecules may be thought of as co-substrates, although for historical reasons they are often known as *coenzymes*! (You will find both the terms coenzyme and group-transfer molecule used in this Course.) They are very much smaller than enzymes, and built up from quite different components as we shall discuss shortly.

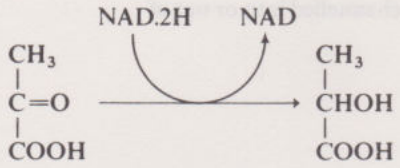
coenzyme

Because a group-transfer molecule is concerned with transferring groups from one substrate molecule to another, it must be able to take part in two types of reaction, one in which it *receives* a group of atoms from a particular substrate, and one in which it *donates* this same group to a different molecule. Without this second reaction, the group-transferring capacity would be permanently blocked. An example should make this clearer. In the early stages of glucose breakdown (as you will soon see) a group-transfer molecule which we shall call NAD removes a hydrogen atom from each of two interacting compounds, which we shall simply call A and B.

$$A + B \xrightarrow{NAD \rightarrow NAD.2H} \text{products} \qquad (4)$$

* In some reactions there is an intervening stage, in which the group is transferred first from coenzyme to enzyme, and then from enzyme to substrate.

By receiving these two hydrogens, one molecule of NAD becomes converted to NAD.2H. It is regenerated to NAD in a second reaction, which involves one of the simple carboxylic acids you met in Unit 23—pyruvic acid. Here NAD.2H passes its hydrogen on to pyruvic acid, as shown in equation 5.



(5)

You will come across NAD again later; for the moment it is important to realise simply that these two reactions are catalysed by *different enzymes*. For group-transfer molecules to act as they do, it is therefore essential that large numbers of quite different enzymes have active sites able to accommodate the same group-transfer molecule.

What is the structure of these group-transfer molecules, and how do they 'load up' with small groups of atoms? You have already encountered one such molecule, the nucleotide adenosine triphosphate or ATP (see Section 9.4 of Unit 23). The group it transfers is usually PO_3H_2 or Ⓟ, the outermost phosphate group of the chain (see Figure 3), and in its unloaded form it is then known as adenosine diphosphate or ADP. You have also just met another common group-transfer

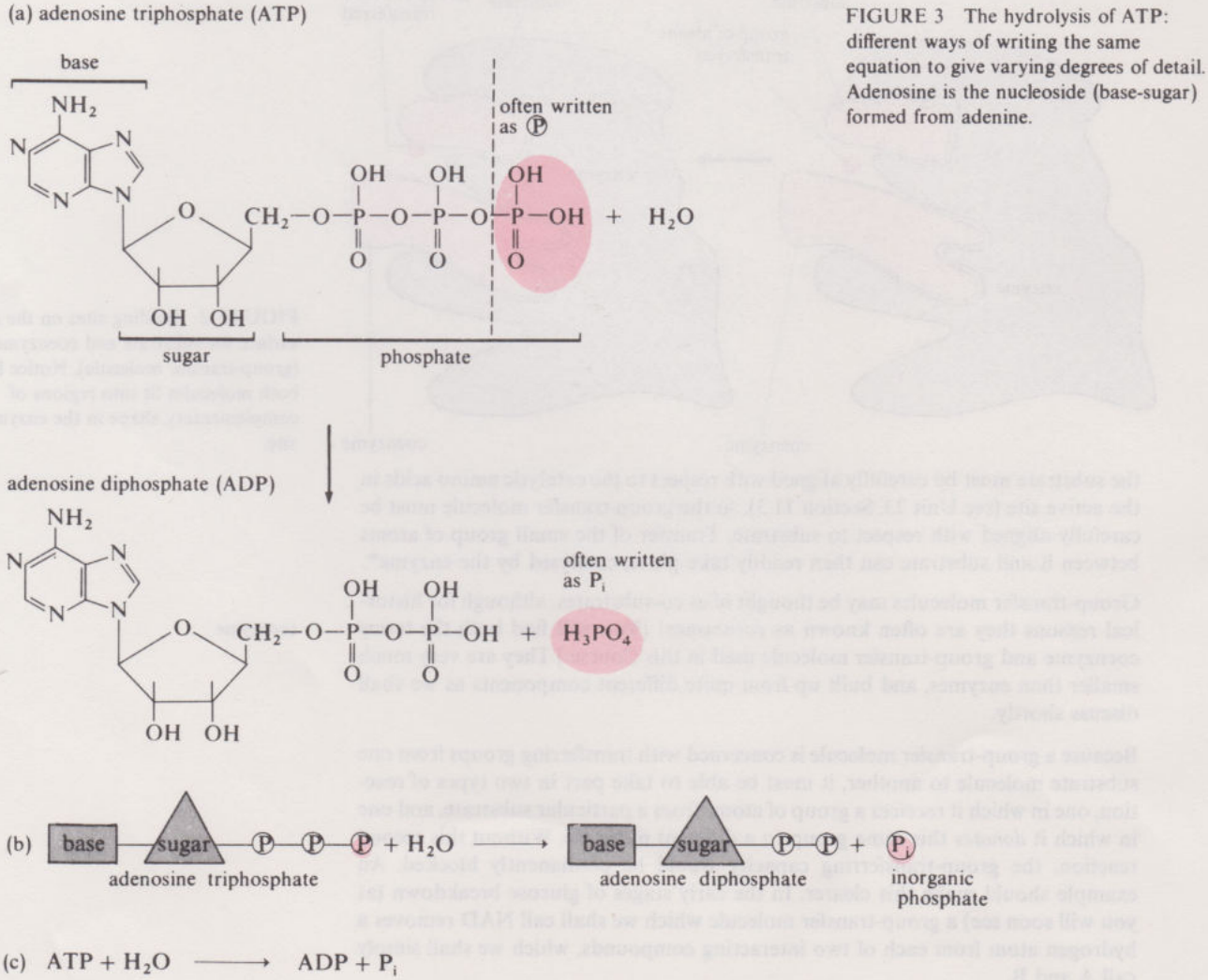


FIGURE 3 The hydrolysis of ATP: different ways of writing the same equation to give varying degrees of detail. Adenosine is the nucleoside (base-sugar) formed from adenine.

molecule, nicotinamide-adenine dinucleotide, which you may remember simply by its initials, NAD. It is rather larger than ATP (being composed of ADP plus another compound) and is involved in the transfer of a much smaller group of atoms—just two hydrogens. These are usually depicted as 2H. The loaded up form of NAD is NAD.2H. A close relation of NAD is NADP, differing from

NAD

NADP

TABLE 2 Group-transfer molecules of the cell

Name of group-transfer molecule		Group of atoms being transferred	Examples of reactions using group-transfer molecules*
Loaded form	Unloaded form		
ATP	ADP	phosphate group, PO_3H_2 or Ⓟ	
NAD.2H	NAD	two hydrogen atoms, 2H	
NADP.2H	NADP	2H	
FAD.2H	FAD	2H	
acetyl Coenzyme A (or acetyl CoA)	Coenzyme A (or CoA)	acetyl group, CH_3CO	
pyridoxamine	pyridoxal	amino group, NH_2	

* This column is left blank, for you to complete on reading Units 24 and 25.

NAD only by the addition of a phosphate group, Ⓟ. It, too, transports hydrogen atoms, but unlike NAD, it tends to participate in biosynthetic rather than degradative reactions. The other group-transfer molecules you will encounter in this Course are shown in Table 2, together with the groups they transfer, and the names of their loaded and unloaded forms. The last column is left blank, for you to fill in as you read through Units 24 and 25.

4.2.1 Vitamins

Although group-transfer molecules are not used up in metabolism, but just shuttled from one enzyme to another, their loss would totally disrupt the workings of the cell. Furthermore, they are put together from fairly complex component molecules, which not all organisms can manufacture for themselves. These component molecules often have to be provided in the diet, and they then take on the status of *vitamins*. In its broad sense, the term vitamin means any component small molecule that is required for the production of macromolecules or group-transfer molecules, but cannot be manufactured by the organism itself. Humans for example, require nicotinic acid* (for making the nicotinamide for NAD), riboflavin (for providing the flavin for FAD), pyridoxal (vitamin B_6), and methionine (an 'essential' amino acid which, like vitamins, cannot be manufactured by the body).

vitamins

4.2.2 Adenosine triphosphate (ATP)

The role of ATP as group-transfer molecule requires further comment. Many of the energy-consuming reactions in biology involve transfer of phosphate from ATP to some other compound. The reasons for this are still not clear, but part of the explanation must lie in its effects on two characteristics of the reaction—*activation energy* (E_a) and *equilibrium constant*.

First let us look at the effect on the equilibrium position. In some reactions, say $A \longrightarrow B$, the equilibrium will lie far to the left, that is, in an equilibrium mixture very little of A will be converted to B. (The reaction $6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6$ is one such example.) Now, if the reaction is altered, say by involving other compounds, we may have $A + X \longrightarrow B + Y$. The effect of involving X and Y is to alter the equilibrium constant (because, after all, we have a new reaction) so that the formation of B is more favoured. In reality, the situation is more complicated than this because reactions in the body are rarely if ever at equilibrium. The key point to remember however, is that involving another compound (ATP, in fact, as we shall shortly see) alters the equilibrium constant so as to favour reaction in a left-to-right direction.

ATP

Now let us look at the effect of ATP on activation energy. As we saw in Unit 15, chemical reactants need to climb an energy barrier before they can be converted to products. This barrier is known as the *energy of activation* and its value depends on the particular reaction taking place. The activation energy for $A \longrightarrow B$ for instance, will be quite different from that for the reaction $A + X \longrightarrow Y + B$. If the second reaction, involving X and Y, has a lower

energy of activation

* Nicotinic acid is also called niacin. Look at the contents description on a packet of cornflakes for examples of these and other essential nutrients.

activation energy than the first, then it is often a more satisfactory route for converting A into B. A similar advantage may be gained by using two consecutive reactions:

$$A + X \xrightarrow{(1)} C;\ C \xrightarrow{(2)} B + Y$$

for the overall conversion $A \longrightarrow B$.

In the cell, reactions with high activation energies are avoided. It is probable that the set of enzymes required to catalyse a simple reaction (glucose + $O_2 \longrightarrow CO_2 + H_2O$, for example) has evolved because there is a great advantage, in terms of activation energy, in forcing the reaction to go through an elaborate series of intermediates. Each small step, converting one intermediate to the next, has a lower activation energy than the reaction in which glucose is oxidized to CO_2 and H_2O in a single step. At several points in this incremental breakdown of glucose, a molecule of ADP is converted to ATP. The ADP/ATP pair is equivalent to the X/Y pair in our side-stepping device, $A + X \longrightarrow Y + B$. Many biological reactions which, like glucose $\longrightarrow CO_2$, we know to take place with the release of energy, are accompanied by the formation of ATP, that is, two processes appear to be going on simultaneously. Biologists speak of these processes as *coupled reactions*. One is:

coupled reactions

$$\text{glucose} + O_2 \longrightarrow CO_2 + H_2O$$

the other is:

$$ADP + P_i \longrightarrow ATP^*$$

Conversely, many energy-requiring processes can continue to take place only if ATP is simultaneously converted to ADP. This is why a rough and ready way of estimating the usefulness of a biological fuel compound in providing for the energy-requiring processes of living organisms, is to estimate the number of molecules of ATP that may be formed during its oxidation. Figure 4 emphasizes these points diagrammatically.

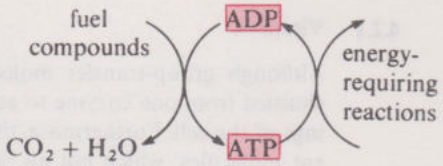


FIGURE 4 The link between energy-requiring and energy-producing reactions.

4.3 Summary of Section 4

1 *Metabolism* is the sum of all chemical reactions in the cell, both degradative and biosynthetic. A *metabolic pathway* is a series of chemical reactions.

2 Degradation of small organic molecule nutrients releases energy. It also provides intermediates for the biosynthesis of other molecules.

3 Chemical reactions in biology seldom occur as simple one-step processes. They tend to take place via a number of small steps, each with a lower activation energy than that of the single-step reaction. Each small step is catalysed by an enzyme.

4 Steps in a metabolic pathway in which groups of atoms are added or removed, often require group-transfer molecules (known also as coenzymes). Both substrate and group-transfer molecule are bound to specific regions in the enzyme active site.

5 ATP is a group-transfer molecule that participates as an intermediate in both energy-producing reactions (which use ADP) and in energy-requiring reactions (which use ATP).

4.4 Objective of Section 4

Now that you have studied Section 4 you should be able to:

(a) Explain what is meant by the following terms: metabolic pathway, biosynthetic and degradative reactions, coenzyme (or group-transfer molecule), coupled reactions.

* The full equation for this second reaction is given in Figure 3. Remember that the P_i stands for 'inorganic phosphate' or H_3PO_4, and Ⓟ stands for the group of atoms PO_3H_2.

SAQ 3 (*Objective* (*a*)) Complete the following paragraphs by inserting the missing words.

ATP is a group-transfer molecule that participates in the enzyme-catalysed reactions of metabolism, by removing a ..... group from one carbon intermediate (the ..... of the enzyme-catalysed reaction) and donating it to another. It could not operate in this way unless, like all ...., it was bound specifically to the .... of more than one enzyme.

By participating in a reaction, ATP may alter both the .... and the .... of the reaction.

SAQ 4 (*Objective* (*a*)) The reaction $A + B{-}NH_2 \longrightarrow A{-}NH_2 + B$ (in which an NH_2 group is transferred from B to A) has an exceedingly high activation energy. If pyridoxamine (see Table 2) participates in the reaction, the activation energy is lowered, and transfer of NH_2 from B to A can readily take place at body temperature. Write down two equations (each with a coupled reaction involving the group-transfer molecule pyridoxamine/pyridoxal) that illustrate this new route for the transfer of NH_2 from B to A.

5 Breakdown of nutrients in the cell

Study comment This is a long, meaty Section, and you should read this Study comment carefully. The simplified diagrams in Figures 5 and 11 you should find helpful for introducing the material, but Figure 6 is the key figure in this Section. (You will find a second copy of it as a looseleaf sheet so that you can have it to hand as you read the whole Section.) Do not be alarmed by Figure 6; you will be expected to remember only those parts of it that are emphasized by shading, etc. (see captions to Figures 7 and 8 for details). You will *not* be expected to memorize the numbers of molecules of ATP and NAD.2H produced (that is, the information in Table 3). But you do need to know where in the cell the three stages of glucose breakdown take place, and in which stages there is production (or utilization) of ATP and reduced coenzymes. You are therefore advised to study Table 3 carefully.

If pressed for time, you may omit Section 5.4.2 on amino acids. Apart from this, you should be familiar with all the information in Table 4.

Having described the principles of metabolism, we can now embark on specific pathways in more detail. Although we shall mention briefly the breakdown of fats and amino acids, this Section is concerned mainly with the breakdown of glucose. Figure 5 summarizes the ground we are to cover and Figure 6 shows it in more detail. In the cell, glucose is broken down in three stages.

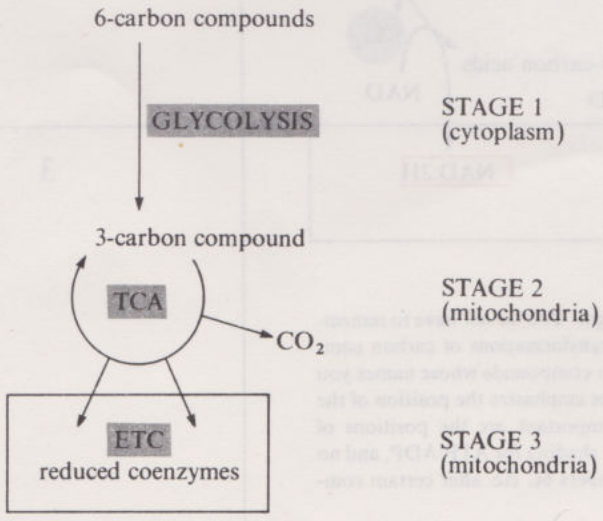


FIGURE 5 Thumbnail sketch of the routes of glucose breakdown in the cell. TCA stands for tricarboxylic acid cycle; ETC for electron transport chain.

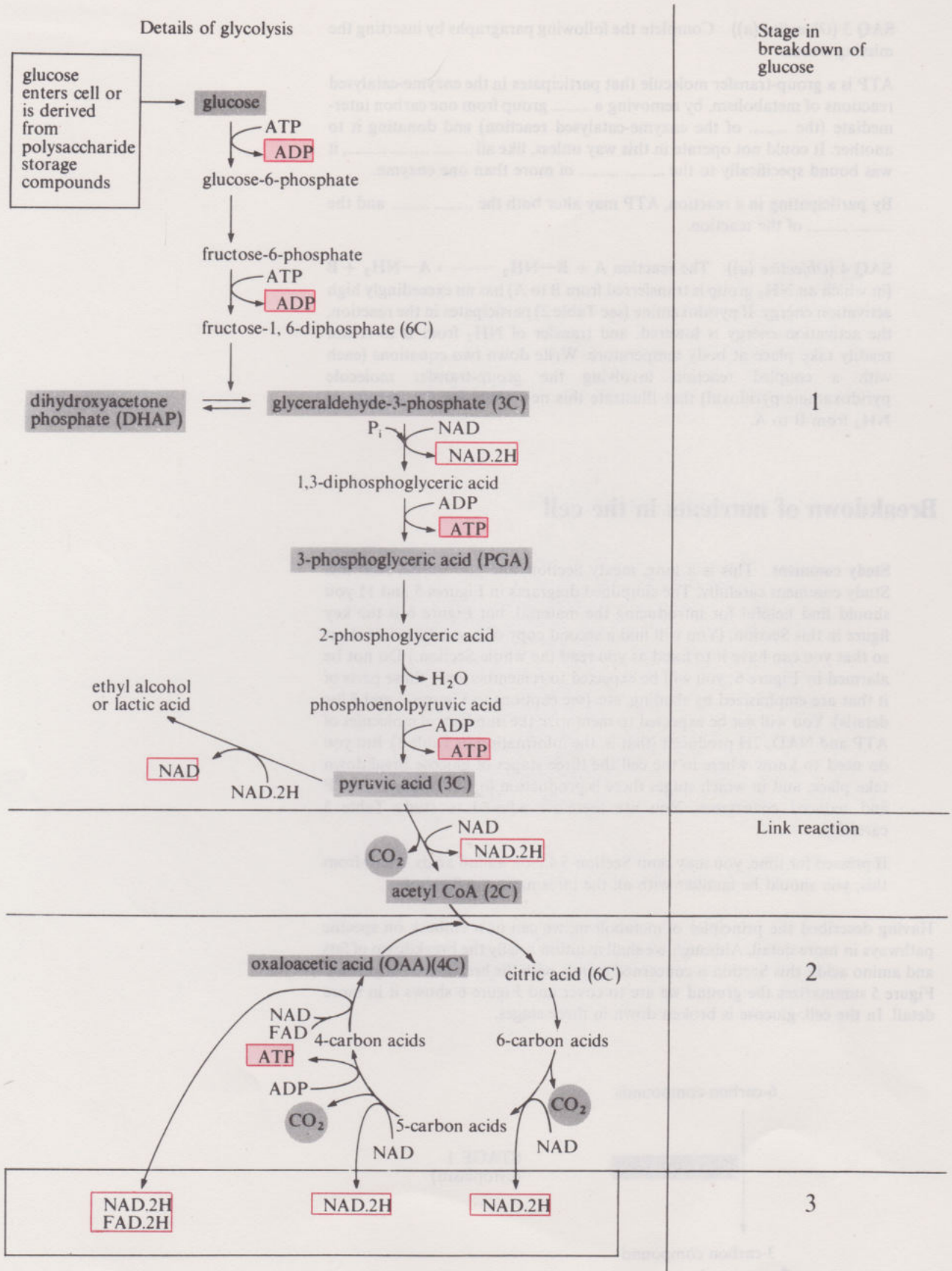


FIGURE 6 Breakdown of glucose in the cell: all three stages. You do *not* have to remember everything in this Figure! You might first trace the transformations of carbon compounds (for example, glucose ⟶ pyruvic acid). Carbon compounds whose names you are expected to remember are in grey rectangles. Grey circles emphasize the position of the final 'carbon intermediate' CO_2 molecules. Particularly important are the positions of group-transfer molecules—these are in red boxes, with pink shading for ATP/ADP, and no shading for NAD/NAD.2H and FAD/FAD.2H. The numbers 6C etc. after certain compounds indicate the number of carbon atoms present.

Stage 1 is *glycolysis* (or 'the glycolytic pathway'), and takes place in the cytoplasm. One molecule of the 6-carbon sugar glucose is converted into two molecules of the 3-carbon compound pyruvic acid. When the cell is well supplied with oxygen, pyruvic acid is further degraded via stages 2 and 3, which take place in a different part of the cell, the mitochondria.

glycolysis

Within the matrix of the mitochondria are the enzymes required for stage 2, the *tricarboxylic acid* (or *TCA*) *cycle*. (This is known also as the Krebs cycle or the citric acid cycle.) It was one of the earliest metabolic pathways to be discovered, and Sir Hans Krebs played a major part in its elucidation. In the TCA cycle, the 3-carbon skeleton of pyruvic acid is broken down into carbon dioxide, and its H atoms are passed to the group-transfer molecules NAD and FAD, forming NAD.2H and FAD.2H.

tricarboxylic acid (TCA) cycle

It is these reduced coenzymes that provide the link between glucose breakdown and oxidation in stage 3, the *electron transport chain* or *ETC*. In the electron transport chain, electrons from reduced coenzymes are passed along a series of carrier molecules to oxygen. Their passage may be linked to ATP synthesis, in a key energy-providing process known as *oxidative phosphorylation*. This produces more molecules of ATP than any other stage of glucose oxidation.

electron transport chain (ETC)

oxidative phosphorylation

5.1 Glycolysis

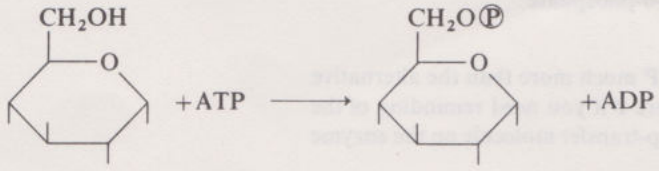
Study comment Glycolysis, the process by which glucose is broken down in the cytoplasm, is shown in Figure 7* which is a repeat of the top part of Figure 6. Individual steps are shown on the left-hand side. On the right-hand side is a simplified scheme, emphasizing the main points. This is the part you will be expected to remember. The left-hand side will be referred to at certain points in the text; the only compounds you will be expected to remember by name are the carbon intermediates that have been shaded grey.

Let us see first what happens to the *carbon skeleton* of glucose during glycolysis. The first four intermediates in the breakdown pathway (including glucose), are all 6-carbon sugars. It is not until the fourth step that the carbon chain is broken to give two 3-carbon compounds. These are interconvertible, so from this point onwards, the entire degradation, right down to and including the ETC, happens twice for each molecule of glucose being degraded. Therefore Figure 7 shows only half the number of molecules of ATP, and half the number of molecules of reduced coenzyme, that are actually produced per molecule of glucose. You will need to remember this when summing up the energy gain per molecule of glucose oxidized. In the second part of the glycolytic pathway, the 3-carbon compounds produced from fructose-1,6-diphosphate** (the last of the 6-carbon intermediates) are converted via four intermediates to two molecules of pyruvic acid.

To study the energy transformations that accompany the breakdown of glucose this far, we must now look at a parallel set of chemical transformations—those concerning group-transfer molecules. (These are indicated in red in Figure 7.) For instance, in the first part of the pathway (the interconversions of different 6-carbon sugars), there are two reactions which need phosphate. In each case this is provided by the group-transfer molecule ATP. Look now at the details of one of these reactions, the phosphorylation of glucose to glucose-6-phosphate (equation 6).

glucose + ATP ⟶ glucose-6-phosphate + ADP

or



(6)

* You are *not* expected to memorize this Figure! The caption suggests how you should deal with it.

** It has recently been recommended that fructose-1,6-*di*phosphate be renamed fructose-1,6-*bis*phosphate (as in ribulose bisphosphate, described later).

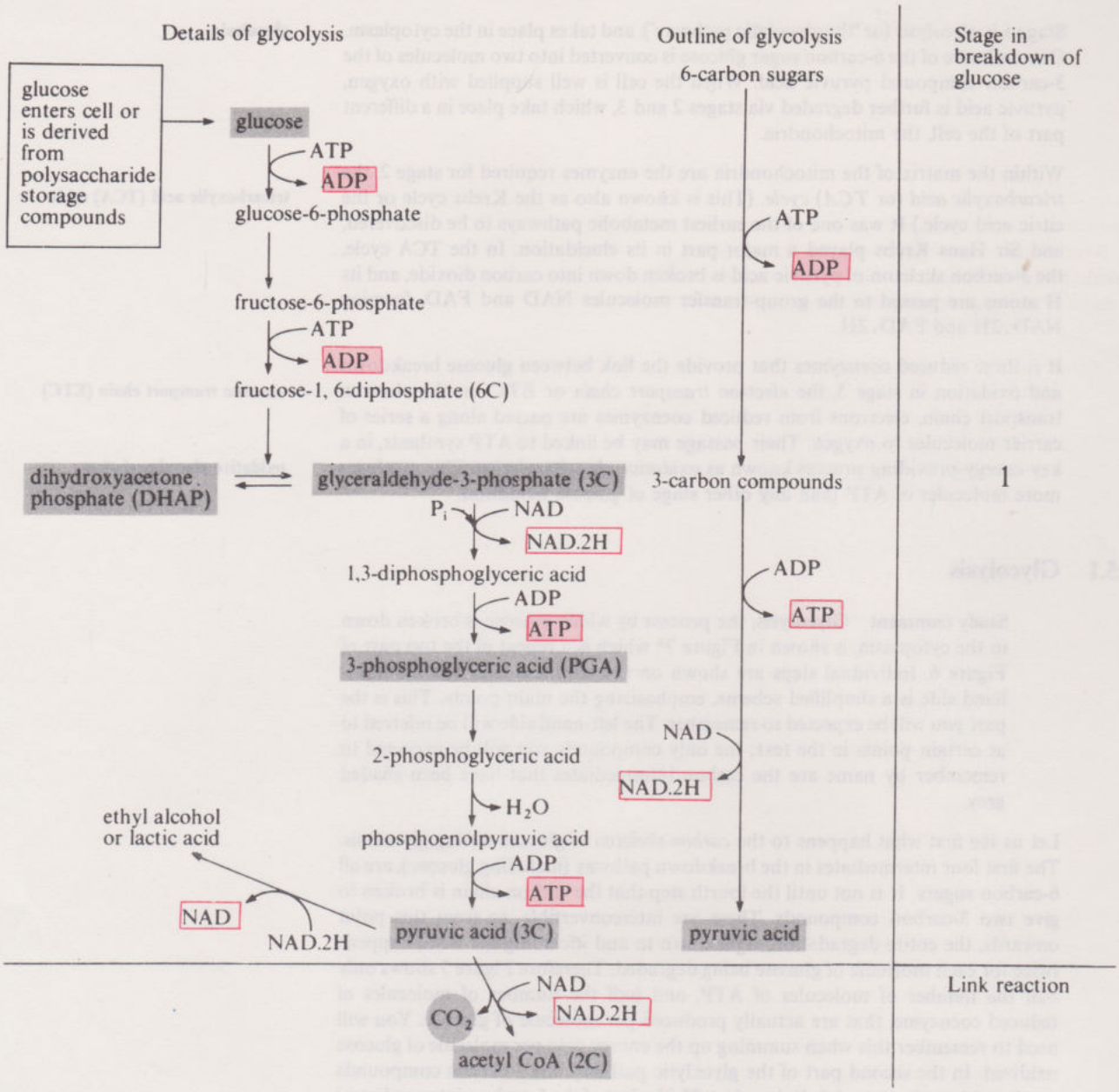


FIGURE 7 Breakdown of glucose in the cell: stage 1, glycolysis. (For colour coding, see caption to Figure 6.)

This reaction is one example of the interaction between phosphate and sugar described in equation 1 of Unit 23 (Section 9.1). The only difference is that there we did not specify the source of phosphate, and here it clearly comes from ATP.

As we said earlier, because the ATP/ADP pair participates in so many different reactions, biochemists tend to think of the phosphorylation of glucose as two *coupled reactions*. This is expressed by rewriting the equation as in equation 7:

$$\text{glucose} \xrightarrow{\text{ATP}\ \text{ADP}} \text{glucose-6-phosphate} \qquad (7)$$

This emphasizes the group-transfer role of ATP much more than the alternative notation shown in equation 6. (Return to Figure 2 if you need reminding of the spatial relationship between substrate and group-transfer molecule on the enzyme surface.)

Although two molecules of ATP lose their phosphates in the earlier part of glycolysis, these are only primer reactions needed to start the pathway off. The cell is more than compensated in the second part of the pathway, which, you may remember, runs twice for each molecule of glucose. Here there are two enzyme-catalysed reactions which involve *removal* of a phosphate from a sugar intermediate. These phosphate groups are passed to ADP, forming ATP again.

Identify from the left-hand side of Figure 7 the two reactions in which ADP is phosphorylated.

1 1,3-diphosphoglyceric acid ——→ 3-phosphoglyceric acid*;
2 phosphoenolpyruvic acid ——→ pyruvic acid.

What is the net gain of ATPs in the breakdown of glucose to pyruvate?

Two. Two are lost in primer reactions in the first part of the pathway; four are gained in the second part, as two molecules of 1,3-diphosphoglyceric acid ——→ PGA, and two molecules of phosphoenolpyruvic acid ——→ pyruvic acid.

Besides ATP, one further type of group-transfer molecule is needed for glycolysis. This is NAD, used in the oxidation of glyceraldehyde-3-phosphate. Summarizing therefore, the changes in carbon intermediates and in group-transfer molecules during the glycolytic pathway can be expressed in the equation:

$$\text{glucose } (C_6H_{12}O_6) \xrightarrow[2NAD \rightarrow 2NAD.2H]{2ADP \rightarrow 2ATP} 2 \text{ pyruvic acid } (C_3H_6O_3) \qquad (8)$$

(*Note* This equation has the colour code used in Figure 6.)

So far, none of the transformations we have just described needs oxygen. They can all carry on under *anaerobic* conditions, that is, in the absence of oxygen. There is however one proviso—the anaerobic breakdown of glucose will grind to a halt, unless there is some means of regenerating NAD from NAD.2H. In the many organisms (like yeast and anaerobic bacteria) and tissues (like white muscle) that regularly operate in the absence of oxygen, pyruvic acid is diverted at the link reaction to give a variety of end products. It is in the conversion of pyruvic acid to these different compounds that NAD is regenerated. In white muscle, the end product is *lactic acid*** (shown as the product in equation 5) and in brewer's yeast, it is *ethyl alcohol*. These three alternative endings to the glycolytic pathway are shown on the left-hand side of Figure 7.

anaerobic conditions

lactic acid
ethyl alcohol

For industrial purposes, yields of alcohol are maximized by growing yeast under anaerobic conditions.

What would happen if the yeast were grown under aerobic conditions?

Glycolysis would stop at pyruvic acid, and the aerobic breakdown stages (that is, TCA cycle and ETC) would take over. Alcohol yields would therefore drop.

Whatever the end product, glycolysis itself releases only a fraction of the energy and of the biosynthetic intermediates that are potentially available from the breakdown of glucose. The full potential is realized only in the presence of oxygen (that is, under *aerobic* conditions) when glucose breakdown goes to completion.

aerobic conditions

Pyruvic acid from glycolysis is then further degraded via the two aerobic stages. These changes are summarized in equation 9.

$$\text{glucose} \xrightarrow[\substack{\text{(glycolysis)}\\ \text{stage 1}}]{\text{(oxygen not needed)}} \text{pyruvic acid} \xrightarrow[\substack{\text{(link reaction + TCA + ETC)}\\ \text{stages 2 + 3}}]{\text{(oxygen essential)}} CO_2 + H_2O \qquad (9)$$

Stages 2 and 3 bring us to a change of location, from cytoplasm to mitochondria.

* We shall abbreviate 3-phosphoglyceric acid to PGA in the rest of this Unit.

** In severe exercise, when the energy requirements are high and glucose breakdown is rapid, lactic acid accumulates in this tissue. It is this build-up of acidity that causes 'cramp'.

5.2 The tricarboxylic acid (TCA) cycle

Study comment This Section is concerned with stage 2 in the breakdown of glucose. Figure 8 (from the lower part of Figure 6) is a key figure.

In stage 2 of glucose breakdown, the carbon skeleton is fully degraded to carbon dioxide, and the glucose H atoms (of which all but two still remain) are transferred to group-transfer molecules NAD and FAD. (See Figure 8.)

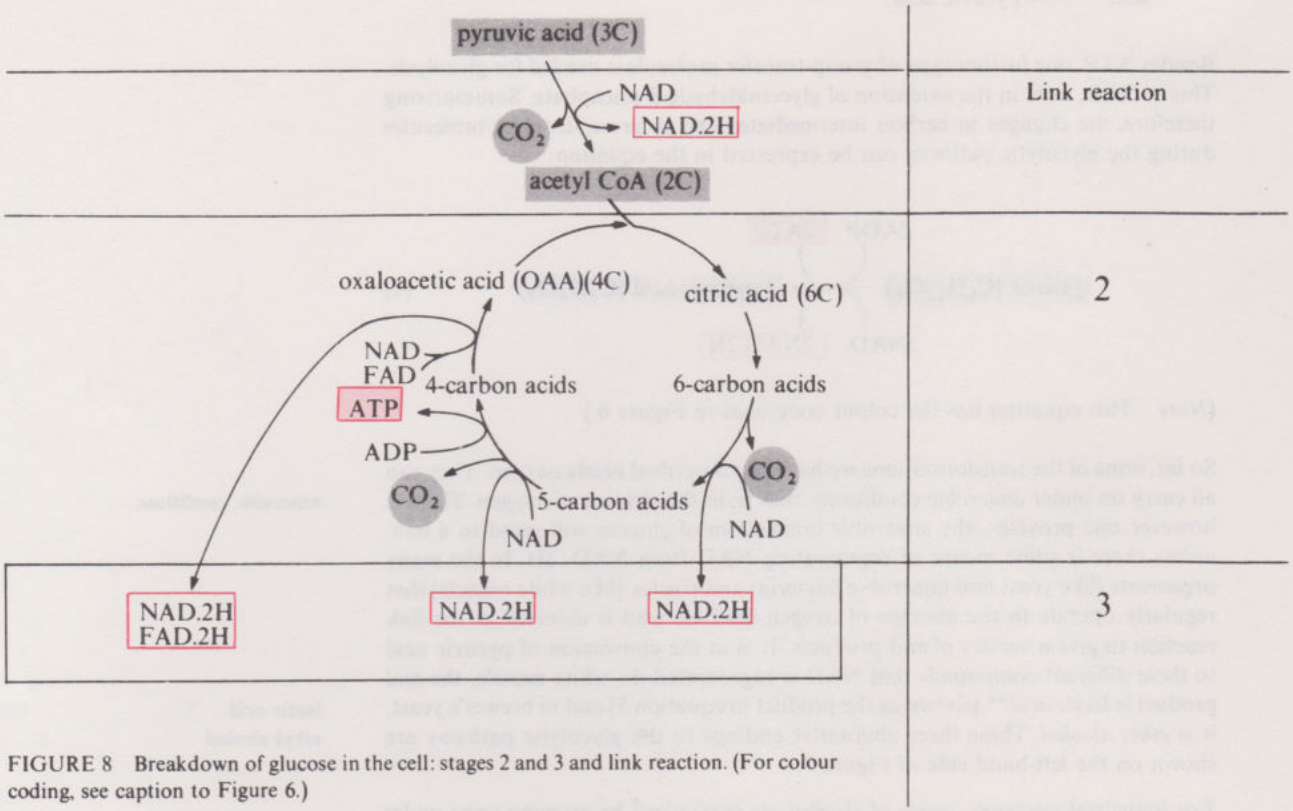


FIGURE 8 Breakdown of glucose in the cell: stages 2 and 3 and link reaction. (For colour coding, see caption to Figure 6.)

We start again by looking at the transformations of the carbon compounds. The most obvious thing about the TCA cycle is that it *is* a cycle. One of the two compounds needed in its first reaction, *oxaloacetic acid* (which we shall now refer to as OAA) is also the product of the last reaction. However the other compound needed in the first reaction is not replenished by the cycle, but has to be continuously fed in from outside. This is the 2-carbon acetyl fragment, CH_3CO. This does not float around the mitochondrial matrix on its own, but is handed from pyruvic acid (the end of glycolysis) to OAA (the beginning of the TCA cycle) by a group-transfer molecule, *Coenzyme A* (*or CoA*). This is acetylated to form *acetyl CoA*, in a complex *link reaction* which joins the end of glycolysis and the beginning of the TCA cycle.

oxaloacetic acid (OAA)

acetyl Coenzyme A (acetyl CoA)

link reaction

This link reaction provides an excellent example of the role of group-transfer molecules in the cell. The pyruvic acid molecule is split into three parts, as shown in Figure 9.

pyruvic acid

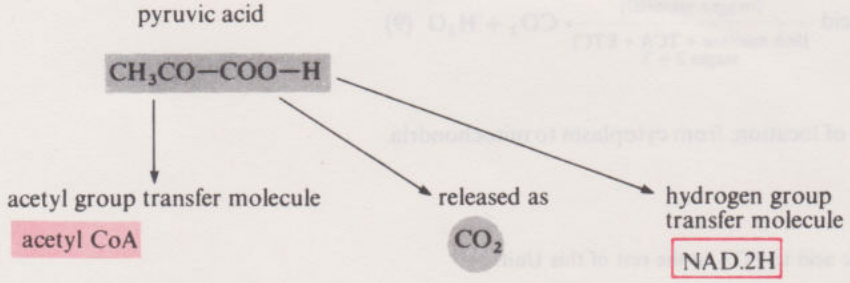


FIGURE 9 Fate of the atoms of pyruvic acid in the link reaction.

Since Coenzyme A can pick up an acetyl group only if it first displaces one of its own H atoms (from its acetyl attachment site), there are a total of two hydrogens

to be transferred to NAD. The overall link reaction may therefore be visualized as equation 10:

Link reaction: $CH_3COCOO\boxed{H}$ → CO_2 (10)

CoA.$\boxed{H}$ → acetyl.CoA

NAD → NAD.$\boxed{2H}$

Not surprisingly, the enzyme which catalyses the impressive collection of reactions in equation 10 is not a single protein, but a closely integrated array of enzymes known as the pyruvate dehydrogenase complex. It is so big that it approaches the size of a small organelle, and can be seen with the electron microscope.

Acetyl Coenzyme A, produced in the link reaction, is an exceedingly important intermediate in the chemistry of the cell. It can pick up acetyl groups from several different sources, and can donate them to a range of intermediates, each of which leads into a different pathway to a different end product. Although we have just described how it is formed from pyruvic acid (and hence ultimately from glucose), it can also come from fats, as we shall see later.

Continuing with glucose oxidation, we now see the acetyl group disappearing into the TCA cycle proper, as acetyl CoA reacts with OAA.

First TCA reaction:

$$CH_3CO{-}CoA + OAA(4C) \longrightarrow CoA + \text{citric acid (6C)} \quad (11)$$

The carbon compounds lying between citric acid and the compound from which OAA is regenerated in the last reaction of the cycle (see Figure 8) need not concern us here in detail. Note only that the two carbons still left from the original glucose molecule after glycolysis and the link reaction are now lost as carbon dioxide, and the H atoms are all passed to NAD or FAD.

Having briefly disposed of the carbon intermediates of the TCA, we can turn to the parallel set of reactions involving group-transfer molecules.

> Use the information in Figure 8 to complete the following summary of what happens to the group-transfer molecules during one turn of the TCA cycle. (In each line, substitute a number for the letter x, y or z.)
>
> x molecules of ADP are phosphorylated to ATP;
> y molecules of NAD are reduced to NAD.2H;
> z molecules of FAD are reduced to FAD.2H.

Answer: $x = 1$; $y = 3$; $z = 1$.

The phosphorylation of ADP in the TCA, like the phosphorylation that accompanies the conversion of 1,3-diphosphoglyceric acid to PGA in glycolysis, does not require direct participation of oxygen. This makes it very different from the other oxygen-dependent ADP phosphorylations which take place in stage 3. We shall leave its details until a later Course, and turn now to the other coenzyme changes accompanying the reactions of the TCA cycle.

In terms of energy transfer, we now come to what is quantitatively the most important step in the breakdown of glucose, the reduction of NAD and FAD.

> If NAD *reduction* is coupled to the transformation of a carbon intermediate, would you expect this transformation to be a hydrolysis, condensation, oxidation or reduction?
>
> An oxidation. As we described in Units 16 and 17, oxidation and reduction are complementary reactions. The H added to *reduce* NAD comes by removing H from the carbon intermediate, which becomes *oxidized*.

The reactions of NAD or FAD in the TCA cycle nearly all involve the kinds of oxidation described in Unit 23. Removal of H atoms from a carbon intermediate either creates a double bond in it ($-CH_2-CH_2 \longrightarrow -CH{=}CH-$) or oxidizes one of its alcohol groups ($>CHOH \longrightarrow >C{=}O$) (see Unit 23, Section 9.2, equations 6 and 7).

We can now sum up all the chemical transformations that take place during stage 2 of glucose breakdown, those of both carbon intermediates and coenzymes.

By replacing the letters *a–g* with appropriate numbers, complete the following summary of events in the TCA cycle, including in your calculations the link reaction with pyruvate.

$$CH_3COCOOH + a\text{NAD} + b\text{FAD} + c\text{ADP} \longrightarrow d\text{CO}_2 + e\text{NAD.2H} + f\text{FAD.2H} + g\text{ATP}$$

Summary equation for link reaction + TCA:

$$\boxed{CH_3COCOOH} + \boxed{4\text{NAD}} + \boxed{1\text{FAD}} + \boxed{1\text{ADP}} \longrightarrow 3CO_2 + \boxed{4\text{NAD}\cdot 2\text{H}} + \boxed{1\text{FAD.2H}} + \boxed{1\text{ATP}} \quad (12)$$

(See Figure 8 if you are unclear about this equation. One NAD.2H and one CO_2 are produced in the link reaction. The rest come from the TCA.)

5.3 The electron transport chain (ETC)

Glucose breakdown is not complete until the reduced coenzymes formed in the TCA cycle, NAD.2H and FAD.2H, have been regenerated for further use, by oxidation. This requires direct participation of molecular oxygen—which is reduced to water. (So here in the ETC we see at last the other two components of the glucose breakdown equation, $C_6H_{12}O_6 + \boxed{6O_2} \longrightarrow \boxed{6H_2O} + 6CO_2$.)

The ETC also requires a slightly different way of looking at the process of oxidation. Up to now we have described it as removal of H or addition of O (while reduction is addition of H, or removal of O).

oxidation (as removal of electrons)
reduction (as addition of electrons)

However, oxidation can also be defined as *removal of electrons*; conversely reduction involves the *addition of electrons**. We shall come across this aspect of reduction in the iron-containing proteins of the electron transport chain. Here iron can be present as iron(II), Fe^{2+}, or as iron(III), Fe^{3+}.

According to the above definition, is the formation of iron(II) from metallic iron oxidation or reduction?

Oxidation, since electrons are *removed* from the metallic iron to form iron(II):

$$Fe \longrightarrow Fe^{2+} + 2e$$

In the iron-containing proteins of the electron transport chain, iron(III) is converted to iron(II), by the addition of one electron, a process which by the above definition is *reduction*.

When we come to describe the oxidation of reduced coenzymes in the ETC, we shall regard the H atoms carried by NAD and FAD as consisting of a proton H^+ and an electron. Once the electron has been removed, the NAD (or FAD) is effectively reoxidized; the H^+ appears to dissociate spontaneously from the coenzyme. Its exact fate need not concern us, but you will see that protons are needed later in the final (oxygen-requiring) step of electron transport.

The reduced coenzymes from the TCA cycle lie in the mitochondrial matrix, where the enzymes for this cycle are located. However, the components of the ETC, where regeneration of coenzymes takes place, all lie in the inner mitochondrial membrane. As you saw in the illustrations of the mitochondrion (see Figure 2 in the Audio-vision Notes for Unit 23) there is an enormous area of contact between membrane and matrix, produced by infoldings of the inner membrane, the cristae. This facilitates transport of reduced coenzymes from their site of production to their site of reoxidation.

* This is an elaboration of the explanation of oxidation and reduction in Units 16 and 17 (Section 2.1).

The components of the ETC lying within the inner mitochondrion include one non-protein component and numerous iron-containing proteins. Among the latter are the cytochromes, proteins which each carry an iron atom in a small *haem* molecule covalently bound to the protein chain. Each component of the ETC is an *electron carrier*. It can exist in an oxidized or a reduced form, the latter containing one more electron than the oxidized form. In the iron-containing proteins like the cytochromes, this electron is carried on the iron atom itself. The reduction step can be expressed as:

$$\boxed{\text{carrier protein} \ldots \text{Fe(III)}} + \text{electron} \longrightarrow \boxed{\text{carrier protein} \ldots \text{Fe(II)}} \quad (13)$$

The ability of a cytochrome to pick up an electron in this way can be quantified; when this is done for all components of the ETC a remarkable fact emerges. The *order* in which components of the chain are arranged in the membrane is approximately the same as the order of their ability to accept electrons. This greatly enhances the efficiency with which electrons are transferred along the chain. When a reduced coenzyme such as NAD.2H, approaches the first member of the chain it becomes reoxidized:

$$\boxed{\text{NAD.2H}} + (\text{two molecules of } \textit{oxidized} \text{ form of electron carrier 1}) \quad (14)$$
$$\longrightarrow \boxed{\text{NAD}} + 2\text{H}^+ + (\text{two molecules of } \textit{reduced} \text{ form of electron carrier 1})$$

Note that *two* electron carrier molecules are needed to regenerate *one* molecule of coenzyme, and that one O atom is equivalent to 2H atoms or two electrons.

You can now follow the passage of the electrons in Figure 10 from one grey-shaded compound to the next. The reduced form of electron carrier 1 passes its electrons on to the next member of the chain, which is lying beside it in the membrane and has a slightly higher electron affinity.

$$(\text{reduced form of carrier 1}) + (\text{oxidized form of carrier 2})$$
$$\longrightarrow (\text{oxidized form of carrier 1}) + (\text{reduced form of carrier 2}) \quad (15)$$

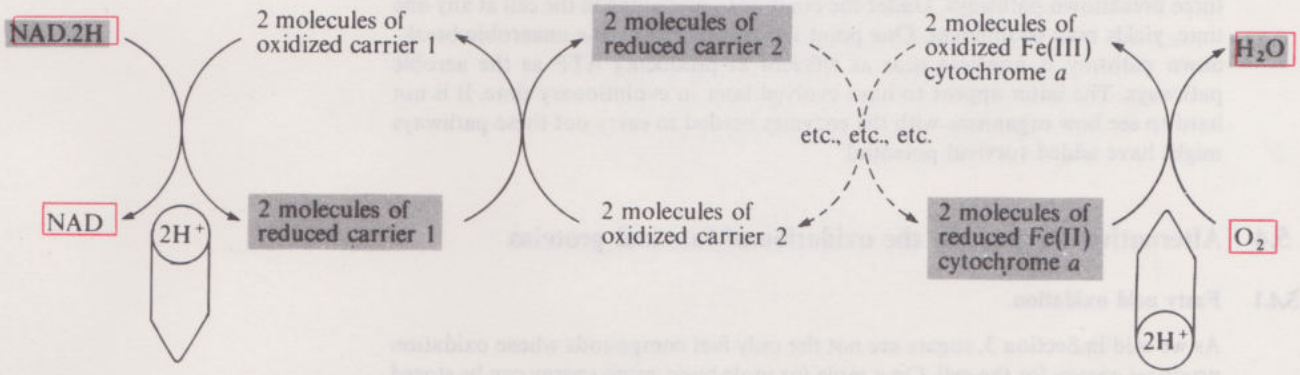


FIGURE 10 Regeneration of NAD by electron transport chain. You can follow the route of electron transfer by the areas shaded grey. Red outline boxes indicate input and output molecules. The words 'etc. etc.' are not a pun on electron transport chain (!) but indicate several intervening electron transfer steps.

In this way, the electrons from NAD.2H—and similarly from FAD.2H—are passed to the last member of the chain. This is cytochrome *a*, which is so avid for electrons that only molecular oxygen can prise them away:

$$(\text{two molecules of reduced cytochrome } a) + \tfrac{1}{2}\text{O}_2 + 2\text{H}^+$$
$$\longrightarrow \text{H}_2\text{O} + (\text{two molecules of oxidized cytochrome } a) \quad (16)$$

Note the reappearance of protons ($2H^+$), equivalent to those removed from NAD.2H in equation 14.

We have now described all three stages in the oxidation of glucose to carbon dioxide and water, but so far we have mentioned the generation of only three molecules of that group transfer-molecule so frequently required in enzyme-consuming processes, ATP. So how is the energy available from glucose oxidation—which we know from measuring the heat output to be quite considerable—actually trapped by ATP formation? This is one of the most interesting questions in cell chemistry today. The process of *oxidative phosphorylation*

(which links coenzyme *oxidation* to ADP *phosphorylation*) is one on which biochemists have expended a great deal of energy (pun!) and much heated discussion. But we shall reserve this story till we come to the chemistry of another energy-transforming organelle, the chloroplast. For the moment we can sum up the energy transformations that accompany the breakdown of glucose with just the following information: passage of electrons during regeneration of one molecule of NAD can be linked to production of *three* molecules of ATP. Similarly, passage of electrons from one molecule of FAD can give *two* molecules of ATP.

Using this information, calculate the number of ATP molecules that could theoretically be produced from stages 1, 2 and 3 in the breakdown of glucose.

Answer: 38. (See Table 3.)

TABLE 3 Coenzyme changes accompanying glucose oxidation

Stage in glucose breakdown	Number of coenzyme molecules converted per molecule of glucose oxidized			
	Number of reduced coenzymes formed	Number of molecules of ATP formed from regeneration of reduced coenzymes	Number of molecules of ATP formed directly	Total number of molecules of ATP formed
glycolysis (glucose → pyruvic acid)	2 NAD.2H	3 × 2 = 6	2	8*
link reaction (pyruvic acid → acetyl CoA)	1 NAD.2H (twice)	3 × 2 = 6	0	6
TCA cycle	3 NAD.2H (twice) 1 FAD.2H (twice)	3 × 6 = 18 2 × 2 = 4	1 (twice)	24

* In anaerobic conditions this figure would be reduced to 2, since the NAD.2H formed during glycolysis could not be regenerated in the ETC.

Although this calculation is extremely useful as a summing-up exercise, we should emphasize that it gives only the *potential* energy-transforming capabilities of the three breakdown pathways. Under the conditions prevailing in the cell at any one time, yields may be different. One point however is clear—the anaerobic breakdown pathway is nowhere near as efficient at producing ATP as the aerobic pathways. The latter appear to have evolved later in evolutionary time. It is not hard to see how organisms with the enzymes needed to carry out these pathways might have added survival potential.

5.4 Alternatives to glucose: the oxidation of fats and proteins

5.4.1 Fatty acid oxidation

As we said in Section 3, sugars are not the only fuel compounds whose oxidation produces energy for the cell. On a mole for mole basis, more energy can be stored when the fuel reserve compound is fat, as in the seeds of higher plants or under the skin of animals.

If the fatty acid in a typical fuel-reserve compound composed of *fat* has the formula $CH_3(CH_2)_nCOOH$, and n is around 16, can you suggest why oxidation of fat can produce more ATPs per molecule than the oxidation of glucose?

The fat has many more H atoms, therefore reduces many more NAD and FAD molecules during its oxidation. (Regeneration of these in the ETC produces the ATP.)

β-oxidation of fatty acids

Oxidation of fats takes place entirely in the mitochondria, beginning with a metabolic pathway known as β-*oxidation*. This is comparable to stage 1 in the breakdown of glucose, and, like glycolysis, it ends in the production of acetyl CoA. Unlike glycolysis, however, it produces not only acetyl CoA, but large numbers of reduced coenzymes for re-oxidation by the ETC. This is where fats score over sugars, as producers of ATP for the energy-requiring processes of the cell.

Since stage 1 of fat oxidation ends in acetyl CoA, the last two stages can follow the same route as glucose oxidation (see Figure 11). The relative importance of glucose and fat as suppliers of acetyl CoA depends on the balance of food reserves in the body. When diet is low in carbohydrate, fat deposits are oxidized instead, hence the rationale of a carbohydrate-free diet for slimmers.

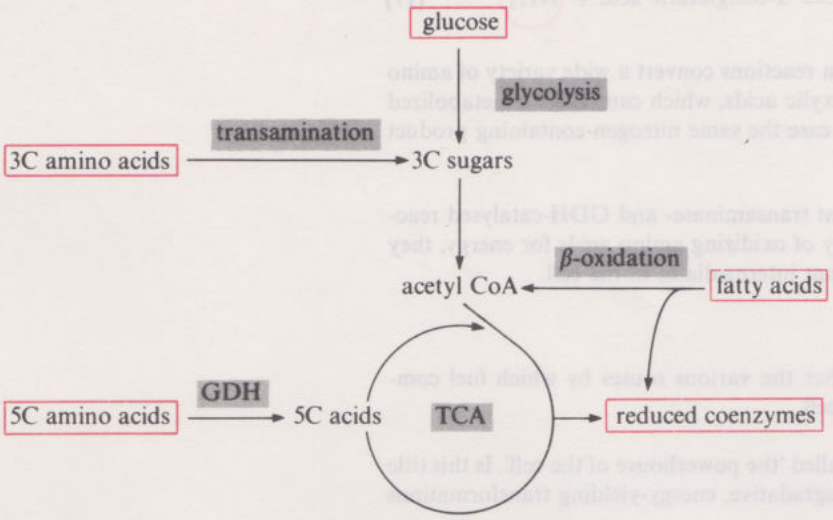


FIGURE 11 Thumbnail sketch showing how amino acids and fatty acids can act as alternatives to glucose as a source of energy (reduced coenzymes) in the cell. Grey shaded areas indicate the principle reactions or reaction pathways involved. Red outline boxes indicate input or output molecules.

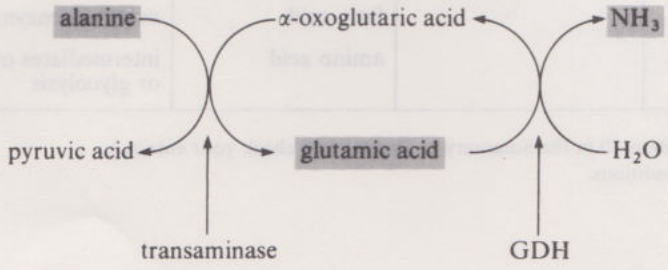
5.4.2 Amino acid oxidation

Another potential source of energy is protein, although this is not its normal function and it is drawn on only in starvation. Remember that, in the gut, protein is hydrolysed back to its component amino acids and these are simply carboxylic acids with amino groups attached. In some cases the carbon skeletons are identical with those of glycolytic or TCA cycle intermediates. For example, we may start with the 3-carbon compound, *pyruvic acid* ($CH_3CO COOH$), formed in glycolysis; this has the same carbon skeleton as the amino acid *alanine*, whose formula may be written $CH_3CH(NH_2)COOH$.

Similarly, one of the 5-carbon acids in the TCA is α-oxoglutaric acid. Adding an NH_2 group turns it into the 5-carbon amino acid, *glutamic acid*.

All that is needed for an amino acid, such as alanine, to be oxidized via the same route as glucose is a *transamination reaction* in which its NH_2 group is removed. The transaminase enzyme catalysing this reaction requires the NH_2-transferring coenzyme, pyridoxal phosphate; this passes the NH_2 group on to another molecule—a carboxylic acid (see Figure 12). It is no accident that the commonest

transamination



or

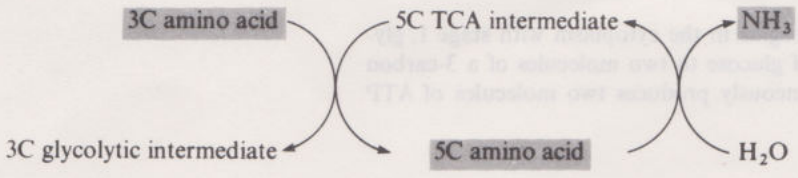


FIGURE 12 Interconversion of amino acids and the intermediates of glycolysis and TCA. You can follow the route of NH_2 transfer by the grey shading.

carboxylic acid on the receiving end of transamination reactions is the TCA intermediate, α-oxoglutaric acid. As we have just seen, adding NH_2 turns this into glutamic acid. The mitochondria are well equipped to deal with this, having large amounts of the enzyme *glutamic dehydrogenase* (GDH). This catalyses the removal of the NH_2 from glutamic acid, leaving α-oxoglutaric acid (which may either

glutamic dehydrogenase (GDH)-catalysed reaction

be used in further transaminase reactions, or oxidized via the TCA). The reaction catalysed by GDH is shown in equation 17, where the two nitrogen-containing compounds are outlined in red.

$$\text{glutamic acid} + H_2O \overset{\text{GDH}}{\rightleftharpoons} \alpha\text{-oxoglutaric acid} + NH_3{}^{*} \qquad (17)$$

In summary therefore, transamination reactions convert a wide variety of amino acids into their corresponding carboxylic acids, which can then be metabolized via glycolysis and the TCA. In each case the same nitrogen-containing product results, glutamic acid.

Figure 12 summarizes these important transaminase- and GDH-catalysed reactions. Not only do they provide a way of oxidizing amino acids for energy, they permit the interconversion of important intermediates in the cell.

5.4.3 Summary: the powerhouse of the cell

The following question brings together the various routes by which fuel compounds may produce energy for the cell.

> The mitochondrion has been called 'the powerhouse of the cell'. Is this title justified? List the number of degradative, energy-yielding transformations that take place here.
>
> Yes. The mitochondrion houses the enzymes for the TCA, the ETC, β-oxidation of fatty acids, transamination and GDH reaction of amino acids. Apart from the early steps of glucose breakdown, it can therefore cope with the complete oxidation of all nutrients, using their H atoms to produce NAD.2H and FAD.2H and hence, via oxidative phosphorylation, ATP.

You should now use the answer to this question to complete column 3 of Table 4, which summarizes much of Section 5.

TABLE 4 Energy from the oxidation of foodstuffs: a summary of the degradative pathways used

Macromolecular foodstuff	Degradative pathways used in oxidation			
	Pathway	Subcellular location*	Starting compound	End product
polysaccharide (e.g. starch or glycogen)	glycolysis		glucose	pyruvic acid**
	TCA		acetyl Coenzyme A (or pyruvic acid)	CO_2 + reduced coenzymes
	ETC		reduced coenzymes	H_2O
fat	β-oxidation		fatty acid	acetyl Coenzyme A
protein	transamination plus GDH		amino acid	intermediates of TCA or glycolysis

* This column is left blank for you to fill in. (See item 10 in the Summary of Section 5 to check your entries.)
** Lactic acid or alcohol, etc., under anaerobic conditions.

5.5 Summary of Section 5

1 Breakdown of glucose in the cell begins in the cytoplasm with stage 1, glycolysis. This converts one molecule of glucose to two molecules of a 3-carbon compound, pyruvic acid, and simultaneously produces two molecules of ATP from ADP.

* NH_3 is the formula for ammonia. Since ammonia (NH_3) is toxic, dealing with it is no mean problem. In mammals, ammonia is converted to urea, which is excreted in the urine. This is why measuring urea levels in urine is one way of monitoring the nitrogen balance of an individual. In starvation, and in the shock that follows surgical operation, the body breaks down muscle and liver protein via the transaminase/GDH reaction, and urea levels in the urine rise sharply.

2 Under anaerobic conditions, glycolysis proceeds one or two steps beyond pyruvic acid to give lactic acid or alcohol, depending on the organism. Whatever the end product, glycolysis releases only a fraction of the energy available from glucose oxidation.

3 In aerobic conditions, breakdown of glucose continues in the mitochondria, via the TCA cycle (stage 2), and the ETC (stage 3).

4 In a link reaction between glycolysis and the TCA cycle, the 3-carbon compound pyruvic acid is converted to a 2-carbon acetyl fragment, CH_3CO, liberating carbon dioxide. The CH_3CO or acetyl group becomes attached to Coenzyme A, forming the important intermediate, acetyl Coenzyme A.

5 In the TCA cycle, the 2-carbon acetyl fragment is converted to carbon dioxide, and the remaining H atoms of glucose are transferred to coenzymes NAD or FAD.

6 The coenzymes reduced in the TCA cycle are regenerated in the electron transport chain of the inner mitochondrion. In oxidative phosphorylation, this regeneration is linked to ATP formation. A maximum of three molecules of ATP may be formed for each NAD regenerated, and two for each FAD.

7 Fats can replace glucose (and other sugars) as fuel compounds in the cell. *Fatty acids* (from fat digestion) are oxidized to acetyl CoA by β-oxidation, a process that produces considerable quantities of reduced coenzymes. This is why fats are more efficient fuel-storage compounds (in terms of the numbers of ATPs that can be formed for each carbon atom converted to CO_2) than sugars (see also SAQ 7).

8 Proteins may also, under certain circumstances, be used as fuel compounds. The NH_2 group of *amino acids* (formed from protein during digestion) passes to glutamic acid and hence to ammonia, via the combined activity of transaminase and GDH enzymes. Depending on its exact formula, the deaminated acid may now act as a glycolytic or TCA intermediate.

9 Stages 2 and 3 in the breakdown of fatty acids and amino acids are the same as the corresponding stages in glucose breakdown.

10 Glycolysis takes place in the cytoplasm. All other stages in the oxidation of glucose, and all stages in the oxidation of fatty acid and amino acid take place in the mitochondria. This includes the regeneration of reduced enzymes from TCA and β-oxidation, by means of the ETC.

5.6 Objectives of Sections 4 and 5

Now that you have studied Sections 4 and 5 you should be able to:

(a) Give examples of the role of group-transfer molecules NAD, ATP and acetyl CoA in the breakdown of glucose, fatty acids and amino acids in the cell.

(b) Compare efficiencies, in terms of the potential production of biosynthetic intermediates, of the aerobic and anaerobic stages in the breakdown of glucose.

(c) Explain the role of reduced coenzymes in the formation of ATP during the oxidation of glucose, fats and proteins.

(d) Describe the three stages in the breakdown of glucose by means of simple diagrams (for example, that in Figure 5), and simple equations (for example, equations 8, 9, 10 and 11).

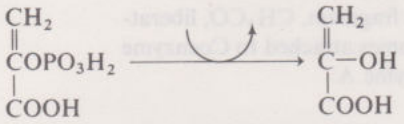
(e) State how many carbon atoms are found in the following compounds: glucose, fructose-1,6-diphosphate, dihydroxyacetone phosphate (DHAP), glyceraldehyde-3-phosphate, pyruvic acid, the acetyl group of acetyl CoA, oxaloacetic acid, citric acid.

(f) Show by means of a simple diagram (like that in Figure 11) how fats and protein could act as alternatives to glucose in the production of ATP.

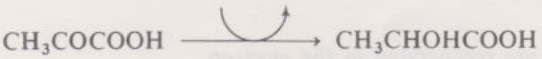
Now test your achievement of these Objectives by attempting the following SAQs:

SAQ 5 (*Objective* (*a*)) Indicate the role of group-transfer molecules in the following equations:

(a) This equation shows the de-phosphorylation of phosphoenolpyruvic acid, during glycolysis:



(b) This equation shows the conversion of pyruvic acid to lactic acid:



SAQ 6 (*Objective* (*b*)) Which of the following intermediates will no longer be available from glucose oxidation, if an organism or a tissue is respiring anaerobically? Glucose-6-phosphate, glutamic acid, pyruvic acid, acetyl CoA.

SAQ 7 (*Objective* (*c*)) One of the principal fatty acids in butter is stearic acid, $CH_3(CH_2)_{16}COOH$. During β-oxidation, this molecule is degraded, 2 carbon atoms at a time. Removal of each such carbon atom *pair* produces *one* molecule of acetyl CoA, *one* of NAD . 2H, and *one* of FAD . 2H.

(a) If these products are both fed into the TCA and ETC, what is the total number of ATPs that could be formed from the oxidation of just *two* of the 16 carbon atoms in stearic acid?

(b) Which process can produce the most ATPs, complete oxidation of the 6C compound *glucose*, or complete oxidation of just *six* of the 16 carbon atoms in stearic acid?

6 Biosynthesis

Study comment Although this Section looks like 'yet more metabolism', it should bring together for you much of what has been said in the previous two Sections. Figure 13 is a central figure; an extra copy is provided as a looseleaf sheet. How you should study it is indicated in the caption. If very pressed for time you could omit Section 6.3 on photosynthesis.

The degradative pathways we have just described serve not only to liberate energy from food reserves, but to form a pool of intermediates which can be used as the starting points of various biosyntheses. In the first part of this Section we shall outline some of the routes by which macromolecules are produced from the small organic molecules of intermediary metabolism. This is equivalent to stage 2 of the food-chain diagram (Figure 1).

We then turn to a more basic question. Where do the raw materials, nitrogen, carbon, etc., come from in the first place? This harks back to step 1 of the food chain, synthesis of small organic molecules from inorganic chemicals. Here we shall briefly discuss the sources of *nitrogen* for living organisms, and then give a slightly more detailed account of *photosynthesis*—the process by which CO_2, in the presence of sunlight, can be used as a source of *carbon* compounds.

biosynthetic pathways
degradative pathways

Although details of these biosynthetic pathways are largely left till later Courses, you should remember that just the same basic principles apply as in a degradative pathway. Chemical transformations take place via a large number of small steps each catalysed by a specific enzyme. However, no biosynthetic pathway is simply a reversal of a degradative pathway. Two totally different routes may operate, as in the synthesis and breakdown of fatty acids. Alternatively, the majority of steps may be simple reversals of degradative reactions, but certain key control reactions

are forced to go via a different route. This is what happens in the reversal of glycolysis. Because of this distinction between biosynthetic and degradative pathways, the two processes can always be regulated separately.

6.1 Building macromolecules from the intermediates of breakdown pathways

Because of the way metabolic pathways interlock, there are many possibilities for converting the molecules derived from one macromolecular foodstuff into those needed to synthesize another. Before examining these possibilities, you need to be clear just which components are needed for putting together any one type of macromolecule.

Recall from Unit 23, and from Table 1 of this Unit, the components of (a) protein (b) polysaccharide (c) nucleic acid (d) fat.

(a) Protein is composed of amino acids.

(b) Polysaccharide is composed of monosaccharides.

(c) Nucleic acid is composed of purine and pyrimidine bases, plus ribose or deoxyribose sugars, plus phosphate.

(d) Fat is composed of glycerol and fatty acids.

Fat can be built up from sugars, a fact all too well known to those who like cakes and biscuits. If you look now at Figure 13 you can see that the glycerol part of a fat can be synthesized from dihydroxyacetone phosphate (DHAP), which is an intermediate of the glycolytic pathway and therefore readily formed from glucose. The fatty acid part of a fat can be synthesized from acetyl coA by a process equivalent to the reverse of β-oxidation (the route by which fatty acids are degraded to acetyl CoA in the mitochondria). In contrast to β-oxidation, however, fatty acid *synthesis* occurs in the cytoplasm, and uses a different set of enzymes.

Figure 13 is on page 30.

dihydroxyacetone phosphate (DHAP)

Some of the amino acids needed for synthesizing the *proteins* of higher organisms may have to be provided in the diet, but the carbon skeletons of the more common ones can be synthesized from intermediates of the TCA cycle and the glycolytic pathway. This is because the transaminase and GDH reactions described earlier are readily reversible. Indeed these enzymes are just as important in biosynthesis as in degradation. In Figure 13 we show only two of the amino acids found in proteins—glutamic acid and aspartic acid. You might like to insert a third—alanine, the pyruvic acid analogue which we described in Section 5.4.2.

As you can see from Figure 13, aspartic acid (the amino acid analogue of the 4-carbon TCA intermediate, OAA) has more than one role in biosynthesis. It is also the starting point for synthesis of the nucleic acid *base*, pyrimidine. The nucleic acid *sugars*, ribose and deoxyribose, can be synthesized from commoner sugars like glucose, via glucose-6-phosphate. This is an early intermediate in the glycolytic pathway. Figure 13 emphasizes how these various biosyntheses interlock.

6.2 Sources of nitrogen

Nutritionists are constantly telling us that a well-balanced diet must contain protein. This is because, like all higher animals, we are unable to metabolize nitrogen unless it comes to us in the form of amino acid.

List some of the molecules whose synthesis might suffer in an animal on a protein deficient diet.

Purine and pyrimidine bases are needed for nucleic acids and for certain group-transfer molecules (such as ATP). Amino acids are needed for synthesis of proteins specific to the organism.

Fortunately, other forms of life are able to tap the enormous supplies of gaseous nitrogen with which we are surrounded. The nitrogen-fixing bacteria have been intensively studied because, either alone or in combination with certain leguminous plants (for example, peas and beans) they can convert elemental nitrogen (N_2) into simple organic compounds. Since nitrogen makes up four-fifths of the

nitrogen fixation

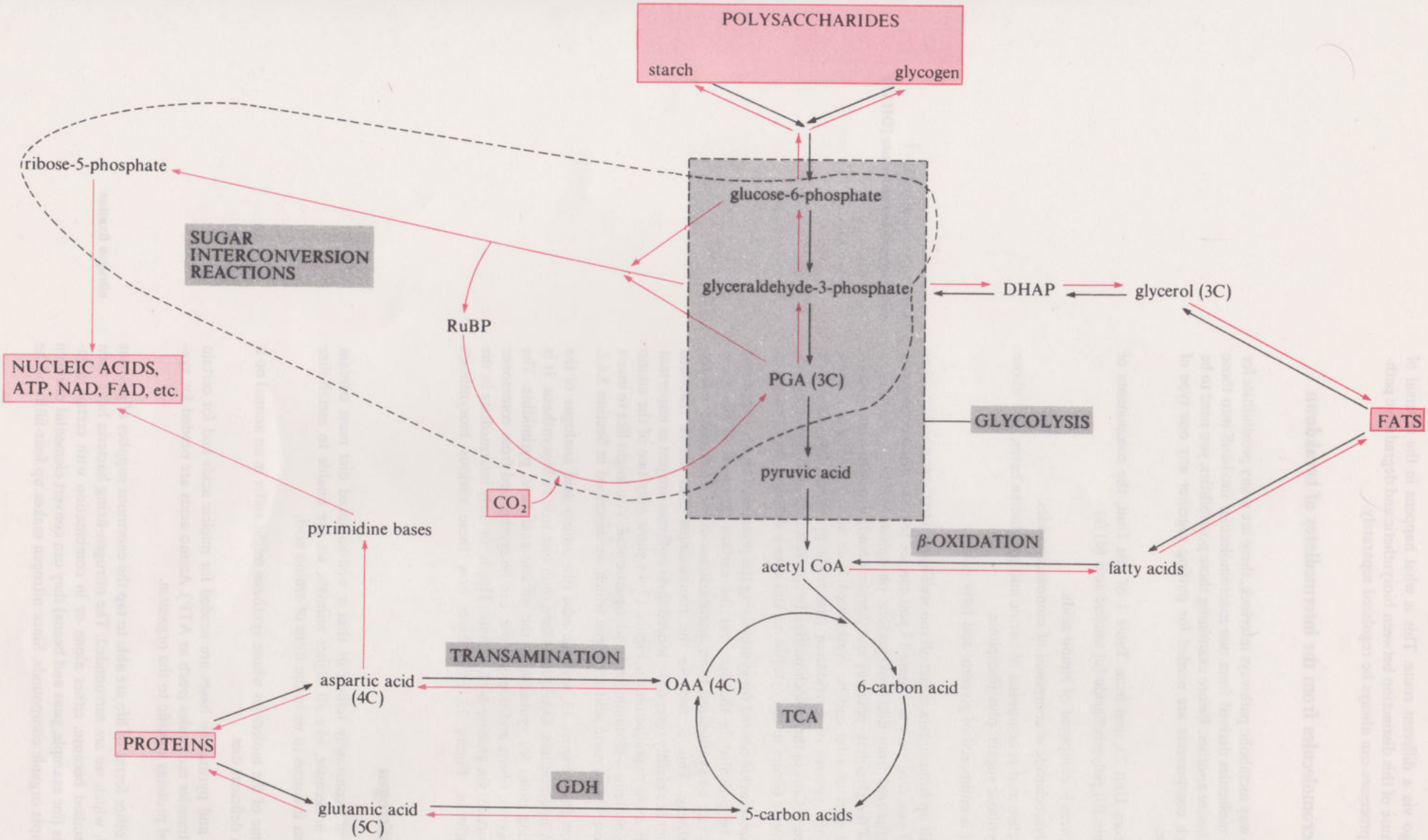


FIGURE 13 Routes for synthesis and degradation of macromolecules in the cell. Again, you are *not* expected to remember everything in this Figure. You should however, remember the input and output molecules (indicated as in Figure 11, by red outline boxes), and the names of principle reactions or reaction pathways involved in their interconversion (grey boxes). The extent of the glycolytic and the sugar interconversion pathways is indicated by dashed lines. You need not remember positions of individual molecules within these pathways.

Note GDH stands for glutamic dehydrogenase, RuBP for ribulose bisphosphate, PGA for phosphoglyceric acid, OAA for oxaloacetic acid.

air we breathe, you can understand the current research interest in these bacteria, and in particular in the genes that code for their 'nitrogenase', the complex enzyme that catalyses nitrogen fixation. Other forms of inorganic nitrogen are nitrate and nitrite ions found in the soil. Many bacteria and higher plants can use these, converting them again into simple organic compounds. (You may recall this from Unit 21.)

6.3 Photosynthesis

Finally we shall describe the events on which the metabolism of all living organisms ultimately depends for both energy and carbon compounds. This is the process of *photosynthesis*, which is restricted to plants and bacteria with the necessary light-absorbing pigments. By far the commonest such pigment is chlorophyll, and in higher plants this is confined to organelles known as chloroplasts, which you studied in Unit 23. Isolated chloroplasts can, on their own, conduct photosynthesis.

If you compare the chemistry of a leaf that is actively photosynthesizing, with one that is prevented from doing so, for example, by being kept in the dark, you will find that photosynthesis is accompanied by the following gross changes: an uptake of carbon dioxide, an output of oxygen and an accumulation of starch (polymerized glucose). As it is a typical carbohydrate, starch may be written as $(CH_2O)_n$. While its carbon atoms come from CO_2, its H atoms have been shown to come from water. This is also the source of the oxygen released in photosynthesis. The overall equation for photosynthesis, comparable to the overall equation for glucose in respiration, may be written as:

$$nCO_2 + nH_2O \longrightarrow (CH_2O)_n + nO_2 \qquad (18)$$

The reaction takes place in two steps, the *light reaction* which can proceed only in the illuminated plant, and the *Calvin cycle* (or *dark reaction*) which can carry on for a short time after the light is switched off.

> Now that you have seen how chemical transformations in the cell proceed through a series of *intermediates*, try suggesting a possible explanation of this effect of switching off the light.
>
> The light reaction uses the energy of the sun to produce *key intermediates*. These are needed in the conversion of carbon dioxide to $(CH_2O)_n$, in a reaction that is not directly dependent on light energy.

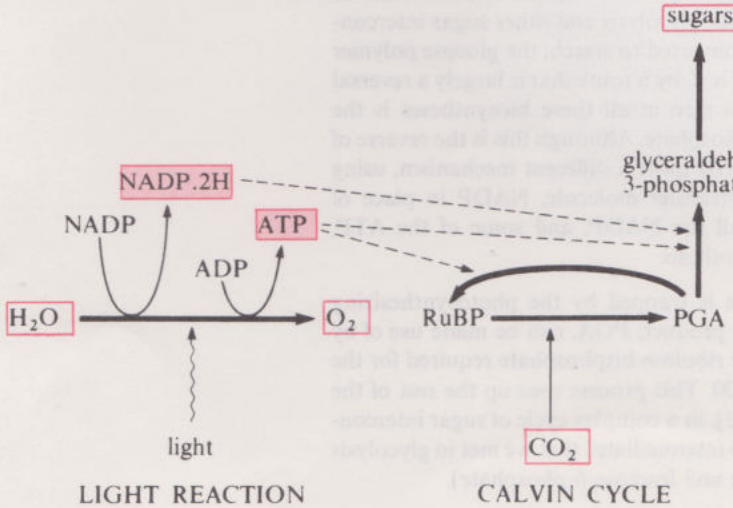


FIGURE 14 Outline of photosynthesis. Red outline boxes indicate input and output molecules, that is, those that appear in equation 18. Pink shading indicates intermediates common to the light reaction and the Calvin cycle.

The key intermediates produced in the light reaction are not simple carbon components, but two group-transfer molecules, ATP (from ADP) and NADP.2H from (NADP)*. Between them these provide the energy and the reducing power needed to convert carbon dioxide to $(CH_2O)_n$ in the second part of photosynthesis, the Calvin cycle. Figure 14 outlines the link between these two parts of photosynthesis.

* Like NAD, the coenzyme NADP transfers H atoms between carbon compounds in the cell. It tends, however, to take part in biosynthetic rather than degradative reactions.

6.3.1 The light reaction

In the presence of light, chlorophyll electrons are promoted from a lower to a higher energy level. It is this one event that distinguishes photosynthesis from other biosynthetic reactions in the cell. The rest of the light reaction is concerned with using the energy from these excited electrons to reduce NADP to NADP.2H and to convert ADP to ATP. The whole of the light stage occurs in the stacked membranes of the chloroplasts (see Plate 9 in the Audio-vision Notes for Unit 23).

Reduction of NADP, like the corresponding oxidation of NAD . 2H in the mitochondrial ETC, is best visualized in terms of electrons. Again it occurs via an ETC, some of whose components are cytochromes and other iron-containing proteins similar to those in mitochondria. Again H_2O is involved, but this time its role is to provide electrons to fill the 'hole' left by the excitation of chlorophyll electrons. (Remember that water is one of the reactants in the overall equation (18) of photosynthesis. Oxygen in the equation also comes from water.)

The conversion of ADP to ATP in the light reaction is known as *photo*phosphorylation, to distinguish it from the corresponding process of *oxidative* phosphorylation in the mitochondrion. In the chloroplast, as in the mitochondrion, passage of electrons between carriers in the ETC is somehow coupled to ATP synthesis. We shall return to the mechanism of this coupling in the next Section.

photophosphorylation

In summary, the light reaction of photosynthesis may be written as:

$$NADP + ADP + P_i + H_2O \xrightarrow{\text{Sun's energy}} NADP.2H + ATP + \tfrac{1}{2}O_2 \quad (19)$$

light reaction of photosynthesis

6.3.2 The Calvin cycle

We now move to the Calvin cycle. None of these reactions are directly dependent on light. The key step is the trapping or 'fixing' of carbon dioxide in the reaction:

$$\text{ribulose bisphosphate} + CO_2 \longrightarrow \text{3-phosphoglyceric acid (PGA)} \quad (20)$$

Calvin cycle

Ribulose bisphosphate (often abbreviated to *RuBP*) is a 5-carbon sugar related to ribose. Addition of one carbon from CO_2 produces *two* molecules of the 3-carbon compound PGA. You have already met PGA in glycolysis (Figure 6). It now assumes a new importance, as the first carbon product of photosynthesis, and as an intermediate common to photosynthesis, glycolysis and other sugar interconversion pathways. It can for example be converted to starch, the glucose polymer that accumulates in the photosynthesizing leaf, by a route that is largely a reversal of the upper part of glycolysis. The first step in all these biosyntheses is the conversion of PGA to glyceraldehyde-3-phosphate. Although this is the reverse of one of the steps in glycolysis, it proceeds by quite a different mechanism, using different enzymes and a different group-transfer molecule, NADP in place of NAD. It is this reaction that uses up all the NADP, and some of the ATP, produced in the light reaction of photosynthesis.

ribulose bisphosphate (RuBP)

3-phosphoglyceric acid (PGA)

You have now seen how carbon dioxide is trapped by the photosynthesizing chloroplast, and how the primary carbon product, PGA, can be made use of by the cell. All that remains is to replenish the ribulose bisphosphate required for the carbon dioxide fixation step of equation 20. This process uses up the rest of the ATP from the light reaction (see Figure 13), in a complex cycle of sugar interconversions, which involves some of the same intermediates that we met in glycolysis (for example, glyceraldehyde-3-phosphate and fructose-6-phosphate).

> Where else have we mentioned a cycle of sugar interconversion steps?
>
> In the formation of the 5-carbon nucleic acid sugars (ribose and deoxyribose) from glucose-6-phosphate (see Figure 13).

Although the Calvin cycle takes place in the chloroplasts, not the cytoplasm, it uses several of the compounds found also as glycolytic intermediates—another example of the economy and interlocking of the pathways of metabolism. For example, synthesis of RuBP uses glyceraldehyde-3-phosphate and fructose-6-phosphate. Ribose-5-phosphate, whose synthesis takes place in the cytoplasm,

derives from glucose-6-phosphate as well as from intermediates lower down the glycolytic pathway.

6.4 An overview of metabolism—inputs and outputs

You have now covered enough metabolic pathways to be able to appreciate, at least in broad outline, just what the cell can accomplish in terms of chemical transformations. We may think of biosynthesis as the process of first converting inorganic molecules to small organic ones, and then putting these together to form macromolecules and other specialized cell products. By looking at Figure 13 you should now be able to see where some of the raw materials for these syntheses originate. The inorganic molecules come from soil or air (see CO_2 in Figure 13; nitrogen sources are not depicted here). Raw materials for synthesizing more complex molecules may be supplied by the breakdown of protein, fat or polysaccharide foodstuffs. Alternatively, for self-sufficient organisms* like plants and bacteria, they may come from the small organic molecules that have been synthesized in the cell itself (for example, the PGA formed from photosynthesis).

In introducing biochemistry, we were not able to depict the cell as anything more than a 'black box'. You can now see that the compounds in Figure 13 that are enclosed in boxes may be visualized as the input or output terminals of metabolism. Having read this far, you should now have some idea of the chemistry that goes on inside the black box.

6.5 Summary of Section 6

1 A degradative pathway may be reversed in part, to produce a biosynthetic pathway. However, certain key reactions will always proceed via different routes in biosynthesis and degradation. Different enzymes, coenzymes and carbon intermediates may all be found at these points. (Good examples are (i) PGA $\rightleftharpoons$ glyceraldehyde-3-phosphate (ii) fatty acid $\rightleftharpoons$ acetyl CoA.)

2 Figure 13 summarizes the routes by which macromolecules are synthesized from small organic molecules. It also shows where one inorganic molecule—CO_2—is fed into the metabolic system.

3 Photosynthesis is the process that uses the energy of sunlight to convert CO_2 and H_2O into sugar. It takes place in two stages.

4 The *light reaction* produces two key intermediates (NADP.2H and ATP), which are then used in a light-independent reaction, the *Calvin cycle*. This converts CO_2 into PGA, in a reaction involving the 5-carbon sugar RuBP. The NADP.2H and part of the ATP of the light reaction are needed to convert the product of CO_2 fixation, PGA, to glyceraldehyde-3-phosphate, and thence to other sugars. The rest of the ATP is needed to replenish RuBP. (See Figure 14.)

6.6 Objectives of Section 6

Now that you have studied Section 6, you should be able to:

(a) Indicate which of the following reactions are concerned in the biosynthesis of polysaccharides, proteins, fats and nucleic acids: glycolysis, TCA cycle, β-oxidation, transaminase/GDH reactions, Calvin cycle, sugar interconversion reactions.

(b) Give examples to show how the degradation products of one macromolecular foodstuff may be used to synthesize another type of macromolecule (e.g. polysaccharide ⟶ nucleic acid components, etc.)

(c) Give examples to show the differences between biosynthetic and degradative pathways.

(d) Describe the process of photosynthesis in terms of simple figures (for example, Figure 14) and simple equations (for example, equations 18–20).

(e) Explain why the Calvin cycle is dependent on the products of the light reaction.

* Autotrophs (see Unit 21).

Now test your achievement of these Objectives by trying the following SAQs:

SAQ 8 (*Objective* (*a*)) A standard technique for elucidating metabolic pathways involves the use of isotopically labelled compounds. If one atom in a molecule is replaced by a more unusual isotope (for example, if $^{12}_{6}C$ is replaced by $^{14}_{6}C$), the molecule is said to be *isotopically labelled*. Its fate can then be followed through a series of chemical transformations in the cell (A ⟶ B ⟶ C ⟶ D etc.), as each intermediate in turn becomes transiently labelled. In *isotope tracer studies*, a radioactively labelled compound may be added to a cell extract, or infiltrated into the intact cell as for example, by injection into the bloodstream. With this approach it is possible to establish not only whether or not one particular compound can be synthesized from another, but the order in which intermediates of the biosynthetic pathways are interconverted.

isotope tracer studies

Each of the *radioactive compounds* given below is added to a cell homogenate, which is then analysed for other radioactive compounds. Place the *intermediates* listed below into a sequence, reflecting the order in which you would expect them to become radioactively labelled in the cell.

Radioactive compound	*Intermediates*
(i) glucose-6-phosphate	PGA, fatty acid, acetyl CoA, fat
(ii) aspartic acid	6-carbon TCA acids, glutamic acid, OAA
(iii) glucose-6-phosphate	nucleic acid, ribose-5-phosphate, pyrimidine base

SAQ 9 (*Objectives* (*d*)–(*e*)) Identify the following statement as TRUE or FALSE, giving reasons for your answer.

The Calvin cycle of reactions cannot proceed in the presence of light.

7 Coupling electron transport to ATP synthesis

Twice in this Unit we have come across systems that link an energy-producing process—electron transport—with an energy-requiring process—the biosynthesis of ATP.

Where in the cell are these two systems located?

1 In the inner mitochondrial membrane, which links electron transport to ATP synthesis in the process known as oxidative phosphorylation.

2 In the chloroplast membrane, where electron transport and ATP synthesis are linked during the light reaction of photosynthesis in the process known as photophosphorylation.

What is the different origin of the electrons that pass down the ETC in mitochondria and in chloroplasts?

In chloroplasts, the electrons originate from water, and are excited to higher energy levels by the absorption of light energy by the pigment chlorophyll. In mitochondria, the electrons come from the reduced coenzymes that accumulate during the oxidation of glucose and other nutrients.

Just how electron transport and ATP synthesis are linked is still far from clear. In the biochemical world, this question has recently been the centre of much controversy—but one that has stimulated an entirely new approach to the fundamental question of energy transfer in the cell.

The membranes responsible for photo- and oxidative phosphorylation have several features in common:

1 *Composition.* Like all membranes, they contain a high proportion of lipid, that is, fatty material that is poorly soluble in water, and virtually impenetrable to water-soluble compounds. Passage through the membrane is therefore restricted to special channels penetrating the fatty barrier.

2 *Electron transport.* Attached to both inner mitochondrial and chloroplast membranes is a series of electron carriers (including the cytochrome proteins).

3 *ATP synthesis.* In both chloroplasts and mitochondria, electron transport can be coupled to ATP synthesis. In chloroplasts, the protein catalysing ATP synthesis is thought to be embedded in the outer membrane of the stacked discs (see Plate 9 in the Audio-vision Notes associated with Unit 23). The comparable protein in mitochondria resides in the 'knobs' protruding from the inner mitochondrial membrane and visible with the electron microscope (see Plate 2 in the Audio-vision Notes).

We know that if mitochondria are carefully prepared, so that their membrane components still retain the same relationship to one another as they do *in vivo*, they can synthesize up to three molecules of ATP during regeneration of one molecule of NAD, and two molecules during regeneration of FAD. In such preparations, electron transport is said to be tightly *coupled* to ATP synthesis. One process cannot go on without the other. If for example, ATP synthesis is made impossible by removing phosphate, then electron transport also stops. The two processes can be uncoupled by slight damage to the mitochondria during preparation, or by addition of molecules known as *uncouplers*. When uncoupler is present, the energy released during oxidation of reduced coenzymes is dissipated as heat, and even though electron transport still proceeds, no ATP is formed. The hormone thyroxin, which stimulates metabolism and raises body temperature, may act in this way.* Uncouplers act in a similar fashion in chloroplasts.

uncoupler

What is the mechanism of this coupling? After heated discussions lasting well over fifteen years, it is now almost universally agreed that the primary event associated with electron transport is the *pumping of protons* from one side of the membrane to the other—an idea first suggested in 1961 by the British biochemist, Peter Mitchell**. Pumping H^+ ions (protons) continuously to the *same* side of a membrane becomes progressively more difficult as the H^+ concentration builds up there. Normally, a small molecule cannot be accumulated against a concentration gradient†, because of its natural tendency to disperse again by diffusion†. Reversing this tendency requires energy. In energy-transferring membranes, the energy of electron transport is used for this preferential concentrating of H^+ ions on one side of the membrane. So long as the membrane remains impermeable to H^+ ions, electron transport energy can therefore be *conserved* in the form of a transmembrane proton gradient.

proton-pumping (chemiosmotic) theory

concentration gradient
diffusion

How is this conserved energy made available for ATP synthesis? Here we come to an area that is still controversial and highly speculative. In tightly coupled mitochondria (or chloroplasts), it is postulated that H^+ ions pass back across the membrane only through special channels, and that these may be closely linked to ATP-synthesizing proteins in the membrane. Return passage of H^+ ions, it is argued, somehow switches the membrane-bound ATP-synthesizing protein into an active state, that is, one in which it can catalyse the energy-requiring reaction $ADP + P_i \longrightarrow ATP$. Figure 15 shows a tentative outline for these still speculative ideas.

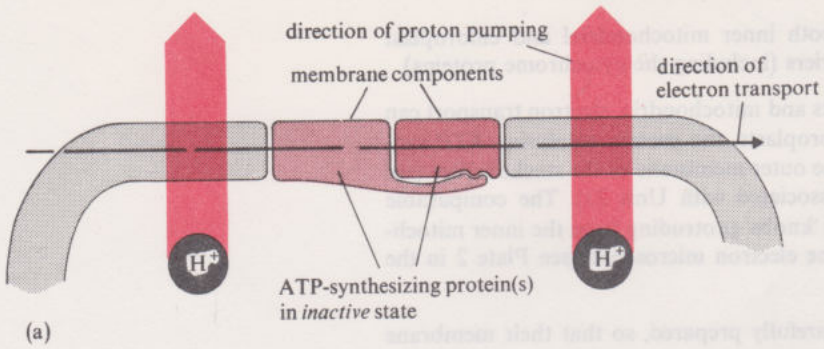
Figure 15 is on p. 36

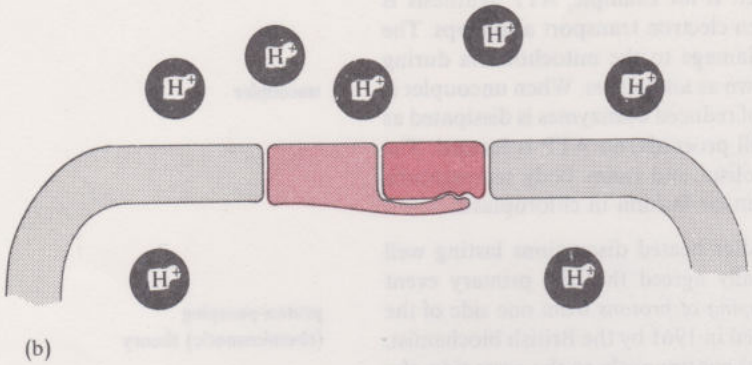
According to the proton-pumping theory, an uncoupler is any compound that causes the membrane to become freely permeable to H^+ ions. It will break the link between electron transport and ATP synthesis. In the presence of uncoupler, return of H^+ ions is no longer restricted to the ATP-synthesizing channels in the membrane, and the energy stored in the concentration gradient is dissipated as heat.

* People with hyperactive thyroids are classically overactive, thin and prone to feeling hot. Certain slimming pills—now illegal because they are dangerous to health—also act as uncouplers.

** As this Unit was going to press (October 1978) we learned that Peter Mitchell had been awarded a Nobel Prize for his proton-pumping (or *chemiosmotic*) hypothesis. This follows sixteen years' work in his small private laboratory in remote Cornwall.

† These terms can be explained by a simple thought experiment. Imagine a spoonful of treacle lying at the bottom of a glass of water. Sugar molecules from the treacle will slowly and spontaneously spread throughout the solution, moving down a *concentration gradient*, that is, from an area of extremely high concentration (the treacle) to one of low concentration (the surrounding water). Movement of molecules down a concentration gradient in this way may be described as *diffusion*. You can imagine that concentrating all the sugar molecules again (that is, reversing the process of diffusion) would require energy.





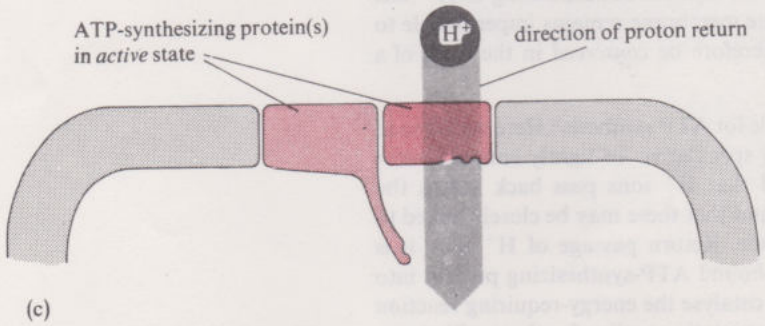


FIGURE 15 Tentative model of the proton-pumping link between electron transport and ATP synthesis in mitochondria.
(a) Electron transport energy is used to pump protons against a concentration gradient.
(b) A transmembrane proton gradient is set up.
(c) Return of protons through special channels activates the ATP-synthesizing proteins.

7.1 Objectives of Section 7

Now that you have studied Section 7, you should be able to:

(a) Explain what is meant by the term *uncoupler*, in relation to oxidative phosphorylation.

(b) Outline how proton pumping is thought to be involved in the coupling of electron transport and ATP synthesis in the mitochondrion.

Now test your achievement of these Objectives by trying the following SAQs:

SAQ 10 (*Objective* (*a*)) Explain, in about two sentences, why a person should lose weight, simply by taking slimming pills that act as uncouplers of oxidative phosphorylation.

SAQ 11 (*Objectives* (*a*) *and* (*b*)) Identify each of the statements (i)–(iii) as TRUE or FALSE, giving reasons for your answer.

(i) Mitochondria are found only in animal cells and chloroplasts only in plant cells.

(ii) Both mitochondria and chloroplasts use an electron transport chain to produce NAD.2H from NAD.

(iii) According to the proton-pumping theory of oxidative phosphorylation, any molecule that renders the inner mitochondrial membrane permeable to protons would uncouple ATP synthesis from electron transport.

Objectives

Now that you have studied Unit 24, you should be able to:

1 Define correctly, recognize the best definitions of, and distinguish between true and false statements concerning the terms, concepts and principles listed in Table A. (SAQs 2, 3, 4, 10 and 11)

2 Explain in terms of the chemical reactions that can be performed by different organisms, why animals are dependent on plants. (SAQ 1)

3 List examples of energy-requiring and energy-producing reactions in the cell. (SAQ 2)

4 Describe in words the chemical changes resulting from the digestion of macromolecular foodstuffs. (SAQ 2)

5 Give examples of the role of group-transfer molecules NAD, ATP and acetyl CoA in the breakdown of glucose, fatty acids and amino acids in the cell. (SAQ 5)

6 Compare efficiencies, in terms of the potential production of biosynthetic intermediates, of the aerobic and anaerobic stages in the breakdown of glucose. (SAQ 6)

7 Explain the role of reduced coenzymes in the formation of ATP during the oxidation of glucose, fats and proteins. (SAQ 7)

8 Describe the three stages in the breakdown of glucose by means of simple diagrams (e.g. Figure 5), and simple equations (e.g. equations 8, 9, 10 and 11).

9 State how many carbon atoms are found in the following compounds: glucose, fructose-1,6-diphosphate, dihydroxacetone phosphate (DHAP), glyceraldehyde-3-phosphate, pyruvic acid, the acetyl group of acetyl CoA, oxaloacetic acid, citric acid.

10 Show by means of a simple diagram (like that in Figure 11) how fats and protein could act as alternatives to glucose in the production of ATP.

11 Indicate which of the following reactions are concerned in the biosynthesis of polysaccharides, proteins, fats and nucleic acids: glycolysis, TCA cycle, β-oxidation, transaminase/GDH reactions, Calvin cycle, sugar interconversion reactions. (SAQ 8)

12 Give examples to show how the degradation products of one macromolecular foodstuff may be used to synthesize another type of macromolecule (for example, polysaccharide ——→ nucleic acid components, etc.)

13 Give examples to show the differences between biosynthetic and degradative pathways.

14 Describe the process of photosynthesis in terms of simple figures (for example, Figure 14) and simple equations (for example, equations 18–20). (SAQ 9)

15 Explain why the Calvin cycle is dependent on the products of the light reaction. (SAQ 9)

16 Outline how proton pumping is thought to be involved in the coupling of electron transport and ATP synthesis in the mitochondrion and in the chloroplast. (SAQ 11)

SAQ answers and comments

SAQ 1 (i) *Photosynthesis.* Plants obtain their fuel-storage compounds (polysaccharide or fat) from the sugars produced in *photosynthesis.* (ii) *Feeding,* followed by *digestion.* Higher animals build up their fuel-storage compounds from the small organic molecules produced by *digestion* of macromolecular foodstuffs. These are derived from plants by *feeding.* (See Figure 1.)

SAQ 2 (a) True.

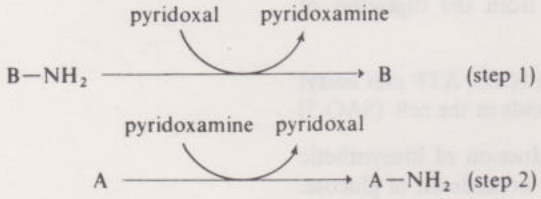
(b) False. Digestion does break down macromolecules, but only to their *organic*, not their inorganic components; it is the reverse of stage 2, not stage 1, in the food chain diagram.

(c) True.

(d) False. Muscular activity is one of the main energy-*requiring* processes in higher animals. The heat produced is only a by-product. (Shivering—the activity of small muscles in the skin—is a means of generating heat, but it too is an energy-consuming process.)

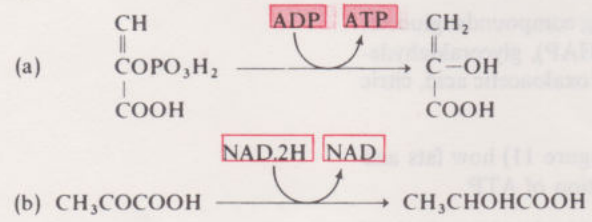
SAQ 3 The missing words are: phosphate; substrate; group-transfer molecules; active site; activation energy; equilibrium constant.

SAQ 4 The overall reaction shown at the beginning of the SAQ now takes place in two steps:



In the first step, the NH_2 is removed from B and transferred to the group-transfer molecule. In the second step, it is transferred to A, forming $A-NH_2$. Both steps will be enzyme-catalysed reactions, in which pyridoxamine has a place in the enzyme active site.

SAQ 5



SAQ 6 Glutamic acid, acetyl CoA (see Figure 8 and equation 18). Glutamic acid will be formed from the TCA intermediate α-oxoglutaric acid.

SAQ 7 (a) 29. (See Table 3, but remember not to include the link reaction itself, and not to double the coenzymes produced in the TCA.)

1 acetyl CoA ⟶ 12 ATPs
1 FAD ⟶ 2 ATPs
1 NAD ⟶ 3 ATPs
17

(b) Complete oxidation of the 6C fragment of stearic acid. This gives 51 ($= 17 \times 3$) ATPs, compared with 38 from glucose.

Now you can see how much more efficient fats are as fuel-storage compounds than sugars!

SAQ 8 (See Figure 13) (i) With radioactive G-6-P, the order of labelling would be: PGA, acetyl CoA, fatty acid, fat.

(ii) With radioactive aspartic acid, the order of labelling would be: OAA, 6C TCA acids, glutamic acid.

(iii) With radioactive G-6-P again, the order of labelling would be: ribose-5-phosphate, nucleic acid. Pyrimidine base would receive very little label, if any, since it is not on the direct route from G-6-P.

SAQ 9 False. The Calvin cycle can proceed at any time, provided the necessary intermediates, NADP.2H and ATP, are there.

SAQ 10 In the presence of uncoupler, electron transport linked to glucose oxidation continues, without the formation of much ATP. Since ATP is essential for many body requirements, more glucose (and other food) has to be broken down than would otherwise be necessary, to provide the essential ATP.

SAQ 11 (i) False. Mitochondria are found in nearly all cells, both plant and animal. Chloroplasts are found only in plant cells.

(ii) False. Both organelles contain an ETC, but in mitochondria this is used to pass electrons from NAD.2H to oxygen. (Therefore NAD, not NAD.2H is produced.) In chloroplasts, NADP.2H, not NAD.2H, is produced.

(iii) True. If the membrane were freely permeable to protons, electron transport energy could not be used to build up a transmembrane proton gradient. Without this gradient, there would be nothing to drive the synthesis of ATP.

S101 Science: A Foundation Course

1 Science and the Planet Earth I

2 Measuring the Solar System

3 Motion under Gravity—A Scientific Theory

4 Earthquake Waves and the Earth's Interior

5 The Earth as a Magnet

6/7 Plate Tectonics: A Revolution in the Earth Sciences

8 Energy

9 Light: Waves or Particles?

10/11 Atomic Structure

12 Chemical Reactions

13 The Periodic Table and Chemical Bonding

14 Chemical Equilibrium

15 Chemical Energetics

16/17 The Chemistry of Carbon Compounds

18 Natural Selection

19 Genetics and Variation

20 Diversity and Evolution

21 Communities of Organisms

22 Physiological Regulation

23 Cell Structure and the Chemical Components of Cells

24 Chemical Reactions in the Cell

25 Genetics: Molecular Aspects

26 Geological Time

27 Earth Materials and Processes

28 Earth History

29 Quantum Theory

30 Quantum Theory of the Atom

31 The Search for Fundamental Particles

32 Science and the Planet Earth II

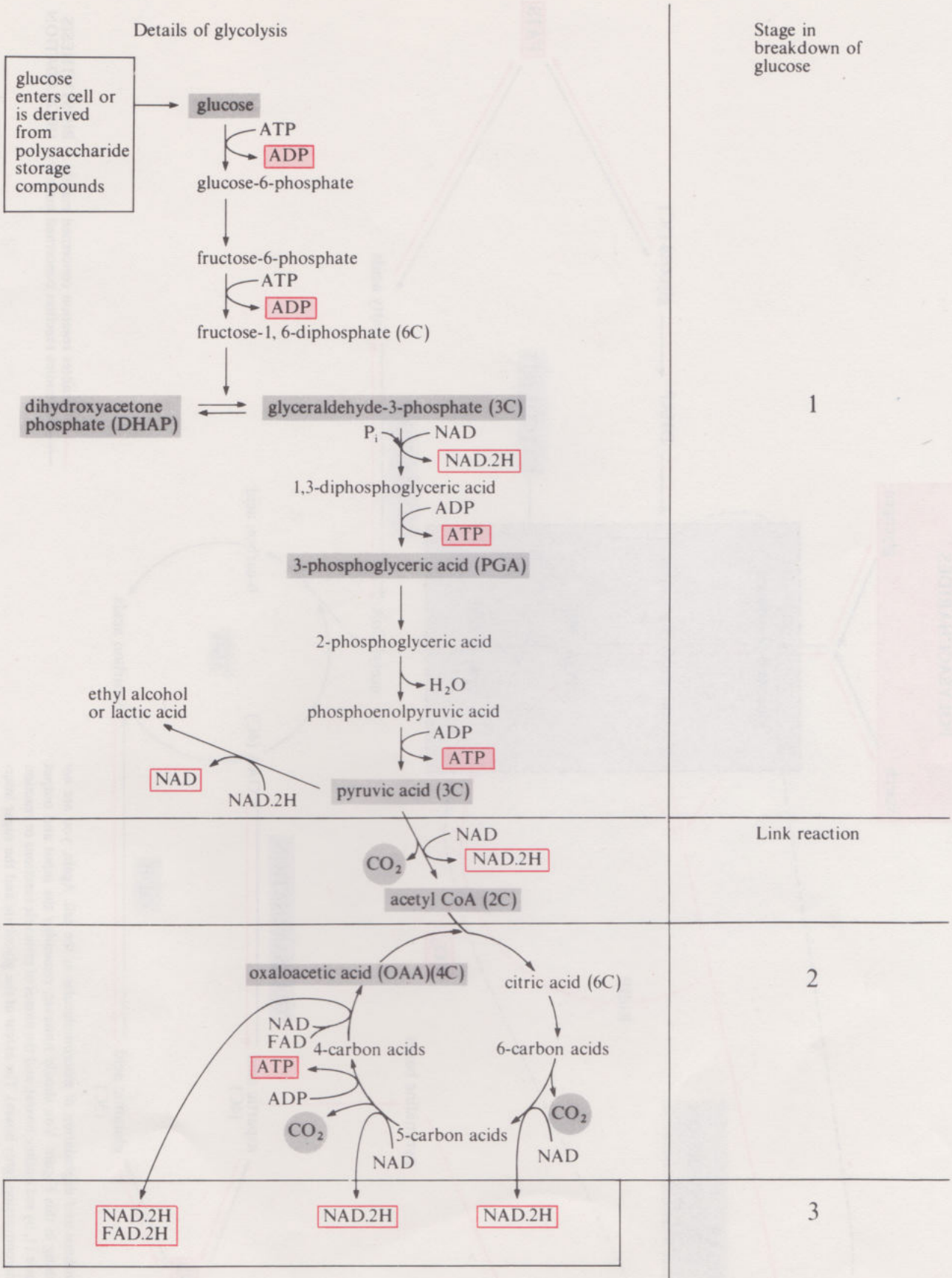


FIGURE 6 Breakdown of glucose in the cell: all three stages. You do *not* have to remember everything in this Figure! You might first trace the transformations of carbon compounds (for example, glucose ⟶ pyruvic acid). Carbon compounds whose names you are expected to remember are in grey rectangles. Grey circles emphasize the position of the final 'carbon intermediate' CO_2 molecules. Particularly important are the positions of group-transfer molecules—these are in red boxes, with pink shading for ATP/ADP, and no shading for NAD/NAD.2H and FAD/FAD.2H. The numbers 6C etc. after certain compounds indicate the number of carbon atoms present.

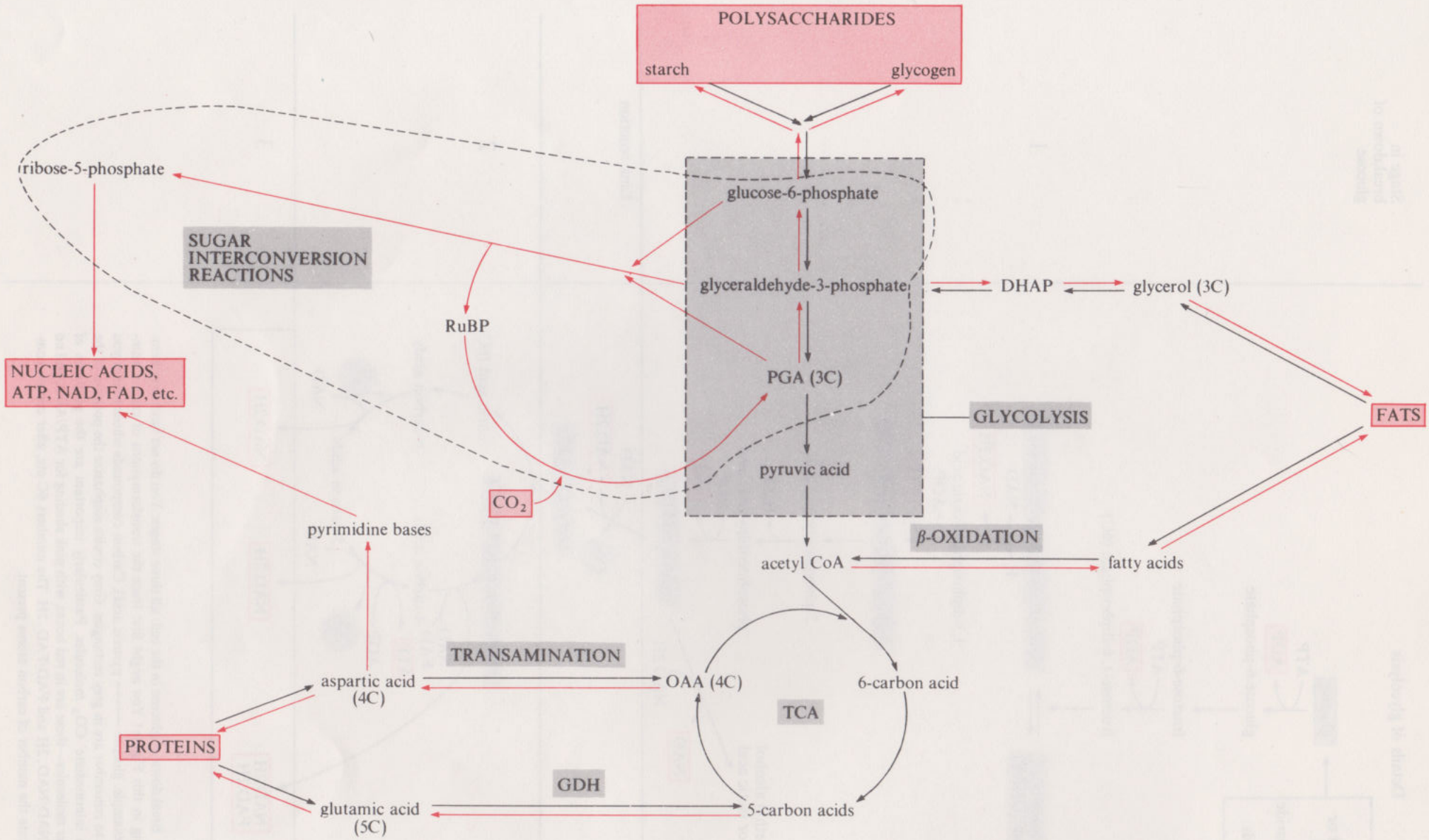


FIGURE 13 Routes for synthesis and degradation of macromolecules in the cell. Again, you are *not* expected to remember everything in this Figure. You should however, remember the input and output molecules (indicated as in Figure 11, by red outline boxes), and the names of principle reactions or reaction pathways involved in their interconversion (grey boxes). The extent of the glycolytic and the sugar interconversion pathways is indicated by dashed lines. You need not remember positions of individual molecules within these pathways.

Note GDH stands for glutamic dehydrogenase, RuBP for ribulose bisphosphate, PGA for phosphoglyceric acid, OAA for oxaloacetic acid.

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